An Investigation of the Potential Influence of Serum Uric Acid Concentration on Regulation of the Human Cardiovascular System

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ABSTRACT

Background

There are strong epidemiological associations between high serum uric acid concentrations and increased cardiovascular risk. However, it is unclear if uric acid is an independent causal risk factor, serves a protective role due to its antioxidant properties, or simply acts as a passive marker of risk through association with other factors. The distinction is important because, if acting as a causal risk factor, treatment to lower uric acid concentrations might reduce cardiovascular risk.

Aims

To study the cardiovascular effects of raising and lowering circulating uric acid concentrations, so as to identify potential mechanisms by which uric acid could impair cardiovascular function or, as an antioxidant, serve a protective role.

Methods

I developed a technique of uric acid administration that allowed the effects of raised concentrations to be examined *in vivo*. The potential impact on serum antioxidant capacity, plasma viscosity, platelet aggregability, systemic haemodynamics, baroreflex sensitivity, large arterial stiffness, and endothelial function were studied in healthy subjects. The effects of high uric acid concentrations were studied in a model of acute exercise-induced oxidative stress, and in regular smokers and patients with type 1 diabetes who are ordinarily exposed to chronic oxidative stress. The effects of lowering uric acid, by means of urate oxidase, were studied in patients with type 2 diabetes to explore whether this might improve vascular function in these patients.

Results

Raising and lowering uric acid concentrations had no effect on vascular function in healthy subjects. Uric acid administration significantly increased serum antioxidant capacity, reduced oxidative stress during acute aerobic exercise, and improved endothelium-dependent vascular responses in regular smokers and patients with type 1 diabetes. Lowering uric acid concentrations did not influence vascular function in healthy subjects or patients with type 2 diabetes.

Conclusions

High uric acid concentrations did not impair vascular function, at least in the acute situation, and appear to preserve vascular function by protecting against oxidative stress in smokers and patients with type 1 diabetes. These findings do not support a causal link between high serum uric acid concentrations and increased cardiovascular risk. Further research is required to define the mechanisms by which high uric acid concentrations ameliorate endothelial dysfunction, and to examine whether these properties have therapeutic potential in diseases characterised by oxidative stress.

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DECLARATION

This thesis represents original research undertaken in the Clinical Pharmacology Unit and Research Centre of the University of Edinburgh at the Western General Hospital. The substantial part of the work described in the thesis is my own, and was performed between 1998 and 2003 whilst working as a Clinical Lecturer in Medicine and based in the Clinical Pharmacology Unit. I am grateful to a number of colleagues that have provided advice and assistance during the research and thesis preparation, and they have been formally acknowledged. The work has been published, in part, in peer-reviewed scientific journals, which are included in the Bibliography section. The contents of this thesis have not been submitted, in whole or in part, for any other degree or postgraduate diploma.

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ABBREVIATIONS

Δ	change from baseline
95% C.I.	95% confidence intervals
ACh	acetylcholine
ADP	adenosine diphosphate
AIx	augmentation index
BP	blood pressure
bpm	beats per minute
°C	degrees Celsius
CI	cardiac index (l/m^2)
BK	bradykinin
BMI	body mass index (kg/m^2)
ECG	electrocardiogram
h	hour(s)
HR	heart rate
kDa	kilo-Dalton
L-NMMA	L-N^G-monomethylarginine
min	minute(s)
mPa.s	milli-Pascal.seconds (1 Pa.s = 1 Newton.second)/m^2)
n	sample size
SD	standard deviation
SEM	standard error of the mean
SNP	sodium nitroprusside
SVRI	systemic vascular resistance index (arbitrary units)
UA	uric acid
UO	urate oxidase

Chapter 1.

Atherosclerosis

1.1 Introduction

Atherosclerosis is the primary cause of cardiovascular disease, and manifests as coronary heart disease, cerebrovascular disease and peripheral arterial disease. At present, cardiovascular disease is the leading cause of death and disability in developed nations, and poses an enormous worldwide public health problem. The global prevalence of atherosclerotic disease is increasing rapidly, and is expected to become the leading cause of death worldwide by 2020 [1]. Current approaches to cardiovascular disease prevention centre on identification and amelioration of major risk factors for atherosclerosis.

1.1.1 Major Cardiovascular Risk Factors

Major risk factors for cardiovascular disease were originally identified in an unselected population enrolled in the Framingham Heart Study. Namely, high total serum cholesterol and low-density lipoprotein cholesterol concentrations, low serum high-density lipoprotein cholesterol concentrations, high blood pressure, regular cigarette smoking, and advancing age [2]. Diabetes mellitus, obesity and family history of premature atherosclerotic disease are also considered major risk factors in their own right [3, 4]. Numerous studies have shown that risk factor modification, including smoking cessation, lowering blood pressure in patients with hypertension and cholesterol reduction in patients with hypercholesterolaemia, significantly reduces the incidence of cardiovascular events and lowers overall mortality.

Ten-year coronary heart disease risk can be estimated on the basis of risk observed in the untreated Framingham study population. Such risk calculations usually take account of age, gender, blood pressure, total serum cholesterol or total: HDL cholesterol ratio, the presence or absence of diabetes, and smoking status. Large studies have confirmed that these factors account for more than 80% of the risk of premature coronary artery disease [5, 6]. Several emerging risk factors have been identified, which bear close associations with cardiovascular disease risk and or the mechanisms involved in the development and promotion of atherosclerosis. These are distinguished from established major risk factors because an independent causal role in cardiovascular disease has not been fully established, and potential clinical

benefits associated with amelioration of these factors has not been demonstrated [7]. Identification of novel risk factors is an important step towards evolving new strategies to prevent or delay the progression of atherosclerosis and thereby reducing cardiovascular risk.

Table 1. Established and emerging cardiovascular risk factors

Major Cardiovascular Risk Factors		
Modifiable	**Life Habit**	**Non-modifiable**
Elevated total cholesterol	Obesity	Advanced age
Elevated LDL cholesterol	Physical inactivity	Male sex
Low HDL cholesterol	High saturated fat	Family history
Diabetes mellitus	intake	
Regular smoking		
Emerging and Potential Cardiovascular Risk Factors		
Inflammatory markers	**Thrombotic factors**	**Platelet factors**
C-reactive protein	Fibrinogen	Platelet aggregation
Interleukins (eg. IL-6)	PAI-1	Platelet size and volume
Vascular and cell	tPA	
adhesion molecules	D-dimer	
Leukocyte count	Fibrinopeptide A	
Lipid-related factors	**Other factors**	
Triglycerides	Metabolic syndrome	Homocysteine
Oxidised LDL	Large arterial stiffness	ACE genotype
Remnant lipoprotein	Blood viscosity	Psychosocial factors
Lipoprotein (a)	Microalbuminuria	

1.2 The Endothelium

In healthy adults, the endothelium is a non-thrombogenic and non-adhesive monocellular layer that covers around 400 m^2, and weighs approximately 1.5 kg and contains an estimated 1.2 trillion cells [8]. The endothelium serves to maintain blood vessel integrity *in vivo*, and acts as a selectively permeable barrier to regulate the passage of macromolecules between the circulation and local tissues. In addition to its structural role as a barrier and transporter, the endothelium is physiologically active and plays a key role in regulating cardiovascular function through the generation and release of a number of paracrine factors.

Table 2. Factors synthesised and released by the endothelium

Vasodilators	Nitric oxide
	Prostacyclin
	Endothelium derived hyperpolarising factor
	Natriuretic peptides
Vasoconstrictors	Endothelin
	Thromboxane A2
	Prostaglandin H2
Pro-thrombotic factors	Tissue factor
	Platelet activating factor
	von Willebrand factor
Anti-thrombotic factors	Tissue factor pathway inhibitor
Fibrinolysis promoters	Tissue plasminogen activator
Fibrinolysis inhibitors	Plasminogen activator inhibitor-1
Leukocyte adhesion molecules	Intracellular adhesion molecule-1
	Vascular cell adhesion molecule-1
	Selectins
Inflammatory mediators	Transforming growth factor
	Tumour necrosis factor
	Chemokines

1.2.1 Nitric oxide

In 1980, Furchgott and Zawadzki observed that vasodilator responses to acetylcholine in isolated aortic rings were dependent on the presence of an intact endothelium [9]. The endothelium was found to release a potent vasodilator in response to acetylcholine, which was originally coined 'endothelium derived relaxing factor' (EDRF), and subsequently characterised as nitric oxide. To date, nitric oxide remains the most extensively studied aspect of endothelial biology. It is formed during a stereo-specific reaction that converts L-arginine to L-citrulline under the control of nitric oxide synthase. Three isoforms of nitric oxide synthase have been characterised: endothelial (eNOS), neuronal (nNOS) and an inducible isoform localised predominantly within inflammatory cells (iNOS). Endothelial nitric oxide synthase is a dimer consisting of two inactive identical monomers, and believed to be principally localised adjacent to the plasmalemmal calveolae, bound to calveolin. The enzyme is inactive in this complexed state, but dissociates in the presence of calcium to become active within the endothelial cell cytoplasm [10]. Hormones and autocrine factors, including acetylcholine, and mechanical factors that stimulate endothelial nitric oxide synthase do so through receptor-mediated increases in intracellular calcium concentrations. Constitutive endothelial nitric oxide synthesis is thought to be evoked by normal calcium concentrations within endothelial cells.

Nitric oxide is a free radical that is rapidly scavenged, for example by haem moieties, so that its effects are observed close to its site of production. It diffuses to the abluminal environment and causes cyclic GMP-mediated decreases in smooth muscle calcium concentration, thereby mediating vascular relaxation. In addition, endothelium-derived nitric oxide inhibits leukocyte adhesion, reduces platelet aggregability and inhibits hypertrophy of underlying vascular smooth muscle. The vasodilating effects of nitric oxide have been demonstrated in epicardial and resistance vessels under normal resting conditions [11]. Nitric oxide has been shown to play a key physiological role in cardiovascular regulation by influencing the contractile state of resistance vessels that determine basal blood flow. Intra-brachial administration of the L-arginine analogue L-NMMA, which inhibits endothelial nitric oxide synthase, is associated with around a 30-40% reduction of resting

forearm blood flow in healthy subjects [12]. Similarly, systemic administration of L-NMMA causes a significant increase in systemic vascular resistance and blood pressure in healthy individuals [13].

1.2.2 Assessment of Endothelial Function

The extent to which regional blood flow is diminished *in vivo* by local administration of nitric oxide synthase inhibitors, such as L-NMMA, is a measure of the extent to which constitutive nitric oxide bioavailability contributes to vascular tone. In states characterised by impaired endothelial function, basal nitric oxide bioavailability is diminished and, therefore, the response to L-NMMA administration is attenuated. Responsiveness of the endothelium to a variety of endothelium-dependent mechanical and pharmacological stimuli can also be examined *in vivo* by measuring regional blood flow, using venous occlusion plethysmography, or assessing the extent of arterial vasodilatation by ultrasonography or angiography.

The forearm vascular bed provides an important model for studying endothelial function *in vivo* because it is readily accessible to minimally-invasive arterial drug administration, and the responses to endothelium-dependent vasoactive substances correlate well with those observed in the coronary circulation [14, 15]. Endothelial function can be determined by measuring the forearm blood flow responses to intra-brachial administration of, for example, acetylcholine. In health, the normal response to acetylcholine administration is vasodilatation and increased blood flow, mediated by the relaxant effects of endothelium-derived nitric oxide on underlying smooth muscle. However, in the presence of severe endothelial dysfunction this response is attenuated, and paradoxical vasoconstriction may be observed due to an unopposed direct effect of acetylcholine on vascular smooth muscle. Concomitant administration of L-NMMA blunts the vasodilator response to acetylcholine confirming that the latter is, at least in part, dependent on endothelium-derived nitric oxide [12]. Administration of endothelium-independent nitric oxide-mediated vasodilator substances, for example sodium nitroprusside, allows assessment of the underlying vascular responsiveness to nitric oxide stimulation. Measurement of blood flow responses to both endothelium-dependent and endothelium-independent

vasodilators takes account of nitric oxide responsiveness, and allows more informed interpretation of endothelial function.

1.2.3 Endothelial Dysfunction

Endothelial dysfunction is associated with impaired nitric oxide bioavailability, and is thought to play an important early role in the development of atherosclerosis. This hypothesis is underpinned by animal models, which have shown that chronic nitric oxide synthase inhibition causes accelerated progression of atherosclerosis and, conversely, that L-arginine supplementation to enhance vascular nitric oxide bioavailability delays development of atherosclerosis [16]. A murine knock-out model has demonstrated that the absence of endothelial nitric oxide synthase expression causes development of atherosclerotic lesions, which are not characteristically seen in the wild type strain [17].

Endothelial dysfunction, characterised by impaired blood flow responses to endothelium-dependent vasodilators, has been identified in patients with any one of a number of major cardiovascular risk factors, including advanced age [18, 19], hypertension [20-22], hypercholesterolaemia [23, 24], cigarette smoking, [25-27], type 1 diabetes [28-30] and type 2 diabetes [31, 32]. Furthermore, the presence of multiple cardiovascular risk factors exerts an additive effect on endothelial dysfunction [24, 33, 34]. Risk factor modification, including cholesterol lowering, smoking cessation, exercise and oestrogen replacement, result in short-term improvements in endothelial function, and are accompanied by long-term improvements in cardiovascular risk reduction. Risk factor modification allows rapid restoration of endothelial function, even in the presence of established atherosclerosis [35], suggesting that vascular nitric oxide bioavailability plays a dynamic role in influencing the development and propagation of atherosclerosis in humans.

1.2.4 Endothelial function as a predictor of clinical outcome

A plausible temporal relationship between the presence of cardiovascular risk factors, endothelial dysfunction and subsequent development of atherosclerosis has been established in laboratory and animal models. The association between risk

factor amelioration and restoration of endothelial function, even in the setting of established atherosclerosis, has stimulated interest in the potential predictive value of endothelial function in a clinical setting. Recently, a number of studies have found that endothelial function carries independent prognostic information in high-risk patient groups, including patients with mild coronary artery disease [36], established coronary artery disease [37], hypertension [38, 39] and peripheral arterial disease [40]. These studies have shown that impaired endothelial function is predictive of increased cardiovascular morbidity and worsened clinical outcome, consistent with animal model data. Therefore, endothelial function has become increasingly accepted as a surrogate marker of future cardiovascular risk. Measurement of endothelial function has become established as an important research tool used to exploring the mechanism involved in development and progression of atherosclerosis, and the potential effects of novel therapeutic agents.

1.3 Large Arterial Compliance

Disruption of endothelium-dependent nitric oxide bioavailability also manifests as reduced large artery compliance [41]. In health, large arterial compliance reduces peak central blood pressure during systole and enhances blood pressure during diastole, thereby minimising the variation between systolic and diastolic blood pressures. Loss of large arterial compliance, so called arterial stiffening, is a characteristic finding in the presence of any one of a number of cardiovascular risk factors, for example diabetes mellitus [42] or hypercholesterolaemia [43]. This is thought due to loss of nitric oxide bioavailability, rather than structural factors alone, and is mimicked *in vivo* by nitric oxide synthase inhibition. Large arterial stiffening is associated with a widened pulse pressure and increased central systolic BP, which is augmented by early pressure waveform reflection from the peripheral arterial tree [43]. This results in increased cardiac work during systole, and reduced coronary blood flow during diastole. There has been increasing interest in the potential mechanisms that promote large arterial stiffness, because the haemodynamic consequences might contribute to increased cardiovascular risk. Recent studies have emphasised the importance of pulse pressure as a more powerful cardiovascular risk predictor than either systolic or diastolic blood pressure alone [45].

1.3.1 Pulse waveform analysis

Pulse wave analysis is an established non-invasive method that allows large artery stiffness to be quantified *in vivo* [46]. Examination of the radial artery pulse waveform by applanation tonometry allows a corresponding aortic pressure waveform to be constructed, using a validated general transfer function [47]. Increased large arterial stiffness causes more rapid waveform propagation, such that reflected waves summate with central pressure waveforms earlier in cardiac cycle, enhancing systolic rather than diastolic pressure. Augmentation index is a measure of the extent to which central blood pressure is augmented during systole by pressure waveforms reflected from the peripheries. Large arterial stiffening is associated with higher pulse waveform velocity and increased augmentation index [48]. Augmentation index is also influenced by the proximity of the effective site of waveform reflection, which is the hypothetical point in the peripheral vasculature from which collective pressure waveforms appear to be reflected towards the aorta. It is governed by structural factors, such as arterial branching points, and sites of significant impedance mismatch. Increased systemic vascular resistance causes impedance mismatch to occur more proximally, at interfaces between conduit and resistance vessels. Therefore, systemic vascular resistance is a potential confounding factor that can influence augmentation index independently [49]. Notwithstanding, augmentation index has been shown to provide a reproducible and reliable measure of large artery stiffness *in vivo* [50-52].

1.3.2 Baroreflex Receptor Sensitivity

The arterial baroreceptor reflex is a key component of the autonomic nervous system, which is normally responsible for regulation of heart rate and central blood pressure. The baroreceptors are stretch-receptive peripheral nerve endings embedded in the adventitia of large arteries, principally located in the carotid sinuses and aortic arch. Action potentials propagate centrally via carotid sinus nerves and the aortic depressor nerve, and synapse with central neurons in the nucleus tractus solitarius of the medulla. These in turn synapse with efferent neurons, which provide parasympathetic and sympathetic innervation of the cardiovascular system.

The viscous and elastic properties of the aorta and carotid arteries are key factors that determine strain on the baroreceptor endings during changes in blood pressure. The baroreceptor reflex is impaired in patients with hypertension and established atherosclerosis, and structural vascular changes resulting in loss of arterial compliance have been considered the predominant mechanisms responsible. Recently, a number of factors have been shown to impair sensitivity and activation of baroreceptors, irrespective of vascular structural abnormalities. These include oxidative stress and free radicals, factors released from activated platelets, and impaired endothelium-dependent prostacyclin and nitric oxide bioavailability [53]. Endothelial dysfunction is capable of impairing baroreceptor activity by effects on humoral factors that directly influence receptor activity, and through loss of large arterial compliance. Therefore, measurement of baroreceptor reflex sensitivity provides an additional means of examining the interaction between endothelial function and large arterial function *in vivo*. Impairment of the baroreceptor reflex mechanism is a recognised finding in patients who have sustained a recent myocardial infarction, and is independently predictive of increased mortality [54].

Conventionally, the baroreceptor reflex has been determined by observing heart rate responses to systemically administered pressor and depressor agents, for example phenylephrine and sodium nitroprusside respectively, and is characterised by the slope of the relationship between change in heart rate and change in blood pressure. However, these manoeuvres examine baroreflex activity during extremes of blood pressure change that may not be physiologically relevant. Furthermore, this method is confounded by direct effects administered drugs might have on baroreceptor function, irrespective of the blood pressure changes they evoke. A more recent approach has been to examine spontaneous baroreflex activity, evident in the patterns of heart rate and blood pressure change encountered under physiological conditions. Sequences of parallel increases or decreases in pulse interval and blood pressure are representative of spontaneous baroreceptor reflex activity, and the slope of the relationship encountered across a number of such sequences has been used to provide a quantitative measurement. This technique, so-called sequence analysis of baroreflex sensitivity, has been shown to be reproducible and reliable in subjects at

rest, and during stimuli known to affect neural control of the cardiovascular system [55]. Similarly, analysis of the relationship between pulse interval and blood pressure variability, using frequency domain rather than time domain analyses, has been established as a reliable means of determining spontaneous baroreceptor reflex sensitivity, so-called spectral analysis [56]. Both sequence and spectral analysis methods allow baroreflex sensitivity to be determined without the need for exogenous drug administration, and across a more physiological range of blood pressure changes than evoked by pharmacological stimulation.

1.4 Tissue plasminogen activator

Considerable attention has been devoted to the impact of endothelial dysfunction on nitric oxide-mediated vascular function. However, there is increasing recognition of the consequences of endothelial dysfunction on other important aspects cardiovascular regulation, including impairment of tissue plasminogen activator activity. Tissue plasminogen activator plays an important physiological role in fibrinolysis by enabling the actions of plasmin. Reduced tissue plasminogen activator concentrations, as a consequence of endothelial dysfunction, confer a pro-thrombotic effect that is believed to enhance the progression of early thrombus formation and atherosclerosis, and contributes to arterial occlusion in acute coronary syndromes [57]. Tissue plasminogen activator concentrations can be measured in forearm venous effluent blood, and the response to intra-brachial administration of specific endothelium-dependent stimuli, for example bradykinin or substance P, can be determined [58, 59]. Administration to localised vascular beds is preferred because it avoids adrenergic activation and central haemodynamic responses evoked by systemic administration of bradykinin, which can independently stimulate tissue plasminogen activator release [60]. Previous studies have found that tissue plasminogen activator release is attenuated in regular smokers, and this is proposed as an important mechanism underlying the increased risk of coronary thrombosis in this group [61, 62]. Furthermore, reduced tissue plasminogen activator concentrations are associated with increased risk in patients with established left ventricular hypertrophy [63], and contribute to ventricular remodelling and congestive heart failure after acute myocardial infarction [64]. Blockade of the renin-

angiotensin-aldosterone by angiotensin-converting enzyme inhibitor treatment increases endothelium-dependent tissue plasminogen activator release, suggesting a possible mechanism by which this class of drugs might lower cardiovascular risk. Tissue plasminogen activator release is an agonist-specific endothelium-dependent response. Muscarinic agonists do not appear to evoke a tissue plasminogen activator response, while the response to substance P is mediated, at least in part, through stimulated nitric oxide production [65]. Bradykinin-mediated endothelial tissue plasminogen activator release appears to be independent of nitric oxide, which might offer advantages to the use of bradykinin as a preferred agonist [59].

1.5 Oxidative stress and cardiovascular dysfunction

1.5.1 Oxidants

An oxidant is any substance capable of removing outer shell electrons from adjacent molecules and, in human biology, free radicals are regarded as the most potent. By definition, free radicals are capable of existing with one or more unpaired electron in their outer shell, and are inherently unstable and highly reactive. Oxygen derived free radicals are of greatest biological significance, including superoxide ($O_2 \cdot -$) and the hydroxyl radical ($OH \cdot$), and normally arise as a consequence of inefficient oxidative metabolism. Molecules subjected to oxidation by free radicals will be left with an unpaired outer shell electron, having become free radicals themselves, and may be capable of propagating further oxidation. In this manner, oxidants can cause severe disruption of cellular function [66].

In the cardiovascular system, lipoproteins are particularly vulnerable to attack, and polyunsaturated fatty acids are susceptible to oxidation at the sites of unsaturated carbon double bonds. Lipoprotein oxidation results in peroxyl radical formation, which in turn reacts with fatty acids to generate lipid peroxides, which are capable of propagating redox chain reactions, and which decompose to form toxic secondary products that directly damage local tissues. Measurement of intermediate and secondary lipid peroxidation products, for example isoprostane and malondialdehyde provides biological markers of oxidant activity within the cardiovascular system [67, 68].

1.5.2 Antioxidants

A number of important physiological antioxidant systems oppose the effects of free radicals and oxidants on target substrates. Those capable of sequestering transition metal ions, which in free form catalyse formation of hydroxyl radicals, can be considered as preventative antioxidants and include the copper-binding protein caeruloplasmin, the iron-binding protein transferrin, and albumin. Enzymatic antioxidants catalyse the breakdown of oxidants within cells, such as superoxide dismutase, catalase and glutathione peroxidase. Non-enzymatic, or 'scavenging', antioxidants are consumed during quenching of free radicals and oxidants, thus preventing organ damage. These sacrificial antioxidants can conveniently be considered as water-soluble or lipid-soluble agents, partly reflecting the respective degree to which each is active in aqueous or lipid-rich compartments. The principal aqueous antioxidants are vitamin C (ascorbic acid) and uric acid, while bilirubin and thiol-containing molecules appear to make a comparatively small contribution [69]. Vitamin C is the most potent electron donor among scavenging antioxidants, and is widely distributed intracellularly and extracellularly, and can be regenerated from its oxidised form, dehydroascorbate. Uric acid is also widely distributed in tissues and extracellular fluid. Circulating uric acid concentrations are significantly higher than those of vitamin C and, therefore, it provides the most abundant source of free radical scavenging activity in human serum.

Vitamin E refers to the tocotrienol group of lipophillic antioxidants, of which α-tocopherol is the most potent. Vitamin E defences can rapidly become depleted, and regeneration requires transfer of electrons from vitamin C at the interface between lipid and aqueous compartments. Carotenoids, including beta-carotene, circulate in lipoproteins and provide a comparatively small contribution to antioxidant activity in man. Ubiquinol-10 (reduced co-enzyme Q10) is present in small quantities and may have an important role in regeneration of active vitamin E.

1.5.3 Oxidative stress

Oxidative stress is a term used to describe an unfavourable imbalance between potentially harmful oxidants and protective antioxidants. Oxidative stress is a

characteristic finding in the presence of major cardiovascular risk factors, including regular smoking, diabetes mellitus, hypertension and hypercholesterolaemia. Oxidative stress in these situations appears to be primarily due to increased oxidant exposure. The presence of multiple risk factors causes a synergistic increase in both oxidant activity [70] and markers of oxidative vascular damage [71]. Regular smokers and patients with type 1 diabetes characteristically have low serum concentrations of ascorbic acid and uric acid [72, 73]. It is unclear to what extent these changes might contribute as a cause of oxidative stress, or whether lower concentrations simply reflect increased consumption in the presence of high oxidant activity. In either case, it is likely that the consequent reduction in antioxidant defences might increase susceptibility to oxidative damage given that both regular smokers and patients with type 1 diabetes are characteristically exposed to excess vascular free radical activity.

1.5.3.1 Isoprostanes

Until recently, the lack of an adequate biological marker of oxidative stress *in vivo* has limited studies of the efficacy of antioxidant supplementation. A number of oxidative reaction products have been explored, including lipid hydroperoxides, malonyl dialdehyde, and prostaglandin F_2-like substances, so called F_2-isoprostanes. F_2-isoprostanes are a family of compounds formed by non-enzymatic oxidative modification of arachadonic acid, and result from free radical attack of phospholipids in cell membranes or circulating low-density lipoproteins. They are formed *in situ* in the cell membrane, from which they are cleaved by phospholipase, and circulate in a free form or as a phospholipid-bound ester, in a bound: free ratio of approximately 1: 2 [68]. Of the F_2-isoprostanes, 8-*iso*-$PGF_{2\alpha}$ ($iPF_{2\alpha}$-III) has been shown capable of exerting a number of biological effects, such as smooth muscle contraction, vasoconstriction, and increased platelet aggregability. However, the relevance of these effects remains uncertain because they are caused by significantly higher 8-*iso*-$PGF_{2\alpha}$ concentrations than typically found in human plasma. Concentrations of 8-*iso*-$PGF_{2\alpha}$ are stable in isolated body fluids, and quantification in plasma or urine provides a marker of free radical generation *in vivo*. Its concentrations in biological fluids are increased in the presence of major cardiovascular risk factors characterised

by oxidative stress, for example hypercholesterolaemia, cigarette smoking, and diabetes mellitus. Elevated 8-*iso*-$PGF_{2\alpha}$ concentrations in these conditions support the hypothesis that oxidative modification is an important early step in the development of atherosclerosis. Circulating 8-*iso*-$PGF_{2\alpha}$ concentrations provide a non-invasive quantitative measure of lipid peroxidation, and represent a major advance in assessment of vascular oxidative stress *in vivo*.

1.5.4 Oxidative stress and endothelial dysfunction

Endothelial cell injury may be a consequence of direct free radical damage, or the result of interactions with toxic secondary products of lipid peroxidation, including oxidised low density lipoprotein. Loss of endothelial integrity is associated with diminished local nitric oxide bioavailability, which predisposes to platelet adherence and release of chemotactic agents. Low density lipoprotein is capable of traversing dysfunctional endothelium, so that cholesterol and cholesterol esters accumulate in the sub-endothelial site. In the presence of oxidative stress, sub-endothelial low density lipoprotein becomes oxidised and acts as a potent chemo-attractant stimulus for macrophage migration and smooth muscle proliferation. Oxidised low density lipoprotein is highly toxic to the endothelium, and thus propagates endothelial dysfunction and accumulation of sub-endothelial low density lipoprotein [74]. Macrophages ingest native low density lipoprotein cholesterol via a receptor-mediated pathway, whereas the oxidised form is ingested by a separate scavenger pathway that is not subject to negative feedback down-regulation. The latter pathway results in formation of cholesterol-saturated macrophages, or foam cells, a characteristic pathological finding in atherosclerotic plaques.

Ordinarily, nitric oxide is subjected to rapid inactivation, and its effects are short-lived and localised to its site of production. Oxidative stress creates an environment conducive to abnormally rapid degradation of nitric oxide, which is accelerated, for example, by oxidised low density lipoprotein [75]. Therefore, oxidative stress is capable of reducing vascular nitric oxide bioavailability through both impaired endothelial production and enhanced oxidative degradation. Increased free radical activity, in the bloodstream or arterial intima, appears to contribute to endothelial

dysfunction, which has stimulated interest in factors capable of reducing oxidative stress, and influencing susceptibility of low density lipoprotein to oxidation.

1.6 Platelet aggregability

Platelets serve an important physiological role by preserving vascular integrity in the absence of injury, and protecting against spontaneous haemorrhage. However, platelet adhesion and mural thrombosis are ubiquitous in the initiation and propagation of atherosclerotic plaques. Platelets are capable of adherence to dysfunctional endothelium and exposed sub-endothelial collagen and macrophages. When activated, they degranulate and release thrombin, cytokines and growth factors that stimulate further platelet adhesion and platelet-platelet interactions, migration of monocytes, proliferation of underlying vascular smooth-muscle cells, and release of pro-coagulant factors [76]. Activated platelets provide a source of free arachadonic acid, which is metabolised to prostaglandins, for example thromboxane A(2), and leukotrienes. Thomboxane A(2) is a potent vasoconstricting and platelet-aggregating substance, while leukotrienes stimulate and amplify local inflammatory responses. Thereby, platelet activation at sites of endothelial damage is believed to play a prominent role in atherogenesis. Studies of induced thrombocytopenia in animal models demonstrate that the absence of platelet activation prevents the development of atherosclerosis [77]. Platelet activation is also a key event initiating event in acute coronary syndromes, whereby platelet activation at the site of coronary plaque rupture promotes additional platelet recruitment and accumulation into an expanding arterial wall thrombus. Platelet or coagulation protein abnormalities have been observed in two-thirds of patients presenting with coronary thrombotic events [78].

1.7 Plasma Viscosity

There has been growing interest in the link between mechanical forces within blood vessels, so-call shear stresses, and the development of atherosclerosis. The development of atherosclerotic plaques is accelerated at sites of low shear stress, typically at arterial bifurcation sites, or where longitudinal forces are diminished by, for example, flow eddies. Shear stress is the energy transferred to the vessel wall due to interaction with fluid in motion, and has been identified as an important stimulus

of endothelium-dependent nitric oxide liberation [79, 80]. Therefore, local nitric oxide bioavailability is reduced under conditions of low vascular shear stress, for example immediately downstream from arterial bifurcation points, and this might in part explain the predisposition to atherogenesis at these sites. It is believed that there is a physiological range of shear stresses, within which diminished shear stress amplifies the risk of atherogenesis, while excess shear stress causes increased vessel wall tension and can stimulate vascular smooth muscle hypertrophy [81].

Three key determinants of shear stress are: cardiac output, vessel geometry (calibre and length), and blood viscosity. Blood viscosity is a measure of the inherent resistance of the blood to changes in flow. Increased viscosity increases drag and enhances the tendency for eddies and low vascular shear stresses, alongside increasing cardiac burden to maintain cardiac output. Blood is a non-Newtonian fluid and, thereby, it will change viscosity dependent on the speed with which it flows. Viscosity at high flow speeds is similar to water, whereas at low flow speeds, for example during diastole, its viscosity increases three to five fold. The key determinants of blood viscosity are plasma viscosity, haematocrit and erythrocyte deformability, fibrin content and, to a lesser extent leukocyte and platelet concentrations. In healthy subjects, plasma viscosity makes the greatest contribution to changes in blood viscosity, given that the other haematological indices are comparatively static. Plasma viscosity is primarily determined by an inverse relationship with temperature, and plasma protein constituents of which large, asymmetric proteins in high concentrations have greatest influence (fibrinogen > immunoglobulins > albumin). Across British populations, plasma viscosity ranges 1.15 - 1.50 mPa.s at 37°C, and there is a close relationship with the risks of ischaemic heart disease [82, 83] and peripheral vascular disease [84]. Plasma viscosity is increased in the presence of any one of a number of major cardiovascular risk factors, including advancing age, male sex, regular smoking, hypertension and hypercholesterolaemia. The effects of these individual risk factors on viscosity is additive, and it has been proposed that changes in blood rheology might offer a potential mechanism by which major cardiovascular risk factors additively increase the risk of arterial disease and atherothrombosis [85]. A causal role is supported by

studies that have identified a relationship between hyperviscosity and increased risk of left ventricular hypertrophy in patients with hypertension [86], and worsened ischaemia in patients with peripheral vascular disease [87]. After myocardial infarction, high plasma viscosity is a strong predictor of 6 month and 2 year mortality [88]. A further study showed that each 0.01 mPa.s increase in plasma viscosity is associated with a 4% increase in coronary heart disease risk [89], and another found that plasma viscosity is strongly associated with the incidence of cardiac events, even after adjustment for the presence of other major cardiovascular risk factors [90].

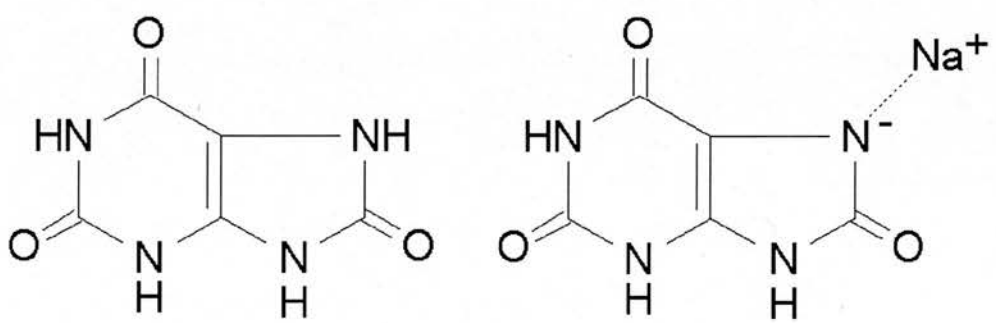
Chapter 2.

Uric Acid

2.1 Physiochemical and Biochemical Properties

Uric acid (2,6,8-trihydroxypurine) can exist in free acid form as a stable, colourless, odourless, off-white powder. Uric acid comprises a five member imidazole ring fused with a six member imidazole ring, and both synthetic and extracted preparations are available with greater than 99% purity determined by high performance liquid-phase chromatography. It is a weak acid that can be ionised at positions 9 and 3, with pKa dissociation constants of 5.57 and 10.30 respectively [91], and confers a modest pH buffering effect in solution. It is comparatively inert, and does not act as a reducing agent under physiological conditions. At pH values greater than 5.57, uric acid exists predominantly in ionised form, as the urate ion, which is more soluble than its free acid. For example, in human serum at pH 7.4, approximately 98% of uric acid exists as monosodium urate, whereas in bodily fluids such as urine with pH less than 5.57, uric acid is the predominant form [92]. This has implications for formation of uric acid-containing crystals in urine in preference to other bodily fluids (discussed further in Chapter 3.5.2).

For the purposes of this thesis, the term uric acid will be used interchangeably throughout to describe either the free or ionised forms, unless otherwise specified.



Uric acid (free form) **Monosodium urate (salt)**

Figure 1. Structural formula of free uric acid and its monosodium salt.

2.2 Uric acid in human biology

2.2.1 Purine metabolism

Nucleic acids derived from dietary sources and endogenous cell turnover comprise purine bases, adenine and guanine, and pyrimidine bases, cytosine, thymine and uracil. In humans, both a *de novo* and a salvage pathway exist for purine biosynthesis, and the liver is the main site of *de novo* purine formation [93]. Certain cells have limited capacity to generate purines, for example neurons, and in some cells the *de novo* pathway may be completely absent, for example erythrocytes. Therefore, the salvage pathway is of physiological relevance, and the nucleotide breakdown products hypoxanthine and guanine are converted to guanosine monophosphate or inosine monophosphate by the action of the enzyme hypoxanthine-guanine phosphoribosyltransferase. Purines participating in the salvage pathway decrease *de novo* synthesis, to some extent, through the negative feedback effects of adenosine monophosphate and guanosine monophosphate on enzyme activity [94]. Purines obtained from dietary sources do not appear to be salvaged but are metabolised by the enzyme xanthine dehydrogenase to uric acid, the ultimate product of purine metabolism in man. The enzyme xanthine dehydrogenase can be converted to xanthine oxidase, an alternative means of purine catabolism to uric acid in humans (discussed further in Chapter 3.2.4). Although purine nucleotides are broken down by all cells, uric acid is only formed in those cells and tissues capable of expressing xanthine dehydrogenase, primarily the liver, small intestine and skeletal muscle.

The rate of uric acid production arising from endogenous purine metabolism in healthy adults is around 400 mg daily, and dietary purine sources contribute to formation of around 300 mg additional uric acid each day [95].

2.2.2 Elimination of uric acid

Approximately 5% of blood uric acid is bound to plasma proteins, and generally regarded as being of little clinical significance [96]. Renal handling of uric acid is complex and involves four sequential processes; glomerular filtration of virtually all of the uric acid entering the glomerulus, reabsorption of 98-100% of filtered uric acid

in the proximal convoluted tubule, secretion of uric acid into the lumen of the distal portion of the proximal convoluted tubule, and further reabsorption from the distal convoluted tubule [97, 98]. Urate reabsorption is indirectly coupled to sodium transport by an electoneutral anion exchanger. Anions, such as chloride and organic acid anions, first enter the proximal tubular cell through sodium-dependent co-transport and move back into the tubular lumen in exchange for uric acid [99]. Urate secretion into the tubule is thought to occur through a voltage-sensitive ATP-dependent transporter mechanism located in the tubular luminal membrane [100]. The net urinary excretion of uric acid is around 6-12% of the amount filtered [97].

2.2.3 Serum uric acid concentrations

Serum uric acid concentration in any individual is determined by the extent of dietary purine ingestion, the rate of endogenous cell turnover and nucleic acid liberation, and the efficiency of renal clearance. Across unselected populations, serum uric acid concentrations observe a Gaussian distribution [101, 102]. Much of the variability results from factors that increase uric acid production (such as high purine or protein diets, alcohol consumption, conditions associated with high cell turnover, or enzymatic defects in purine metabolism) or factors that influence clearance, and renal function in particular [103]. Concentrations are elevated on the first day after birth but drop to a low level in the succeeding days and remain low until approximately the 10^{th} year [104]. Serum uric acid concentrations achieve adult values with the beginning of puberty and a typical 95% normal reference range is 120-420 μmol/l in men and 120-360 μmol/l in women [104]. Exposure to lower uric acid concentrations in women appears due, at least in part, to the effect of oestrogen on renal uric acid clearance [105]. Both supplemental oestrogen therapy and oestrogen replacement therapy cause enhanced uric acid clearance, and reduce serum concentrations. Consistent with these observations, serum uric acid concentrations increase in post-menopausal females, and the differences between men and women decrease with advancing age [106]. Hyperuricaemia is arbitrarily defined by a serum or plasma uric acid concentration of greater than 420 μmol in men or greater than 360 μmol/l in women. Asymptomatic hyperuricaemia is frequently detected by

biochemical screening, and few of such individuals ever develop clinical disorders thought directly attributable to raised uric acid concentrations (see Chapter 3.5).

Uric acid has limited solubility in aqueous media and human bodily fluids. The maximal achievable concentration of uric acid in water is around 380 mmol/l at 37°C, and plasma becomes saturated at uric acid concentrations beyond 405 μmol/l [107, 108]. At higher concentrations, plasma is supersaturated with regards to uric acid, and potential exists for precipitation of uric acid crystals, particularly at concentrations greater than 650 μmol/l [109]. In the uncommon setting of acute tumour lysis syndrome, serum uric acid concentrations of more than 1200 μmol/l have been described [110]. The solubility of uric acid increases as sodium concentration decreases and body temperature increases [109]. A number of drugs are known to influence serum uric acid concentrations [111, 112].

Table 3. Drugs known to influence serum uric acid concentrations.

Increased uric acid concentrations	**Decreased uric acid concentrations**
Thiazide diuretics	Probenecid, sulphinpyrazone
High dose loop diuretics	Allopurinol, oxypurinol
Salicylates (low dose)	Ascorbic acid
Nicotinic acid	Calcitonin
Ciclosporin	Calcium channel blockers
Lead	Oestrogens
Levodopa	Fenofibrate
Ethanol	Glucocorticoids
Ethambutol	Salicylates (> 2 g/day)
Pyrazinamide	ACE inhibitors
Vitamin B12	Losartan
Radiographic contrast agents	Verapamil

2.2.3.1 Circadian variation in serum uric acid concentrations

Circadian variations in cell turnover, metabolism and renal function have long been recognised. These factors can influence circulating uric acid concentrations and,

therefore, circadian patterns of uric acid clearance and serum concentrations have been observed. For example, uric acid clearance has been studied in healthy people during a twenty-four hour period of recumbent rest, under conditions of adequate hydration. The lowest values were observed at night, the steepest rise in uric acid clearance occurred between 7 and 10 am, and there was around a two-fold difference between highest and lowest clearance throughout the study period [113]. Similar circadian patterns of serum uric acid concentrations have been observed in ambulatory healthy individuals, where highest concentrations are observed during wakeful hours, and lowest concentrations during and after sleep [114]. Hour-by-hour variations in serum uric acid concentrations are likely to reflect not only patterns of clearance, but also the effects of dietary intake and exercise. Lactate, produced during ambulation and moderately intense exercise inhibits tubular secretion of uric acid so that serum concentrations increase [115].

2.2.4 Intracellular uric acid concentrations

In healthy individuals, the normal total body pool of exchangeable uric acid is estimated at around 1.2 g in men and 0.6 g in women, and is subject to much greater variation between individuals than that reflected by serum measurements [116]. Transport of uric acid across cell membranes into endothelial cells depends on an active carrier-mediated mechanism transport, and intracellular concentrations are lower than those in the circulation. Furthermore, active cellular efflux, assisted by the negative membrane potential and lower cellular pH, serve to maintain lower intracellular uric acid concentrations [117]. Therefore, high circulating uric acid concentrations may not necessarily be accompanied by correspondingly high concentrations within tissues. The lack of close correlation between circulating uric acid concentrations and those found in other fluids and tissues is an important confounding factor that explains the poor correlation between serum concentrations and the risk of crystal arthropathy (discussed in Chapter 3.5.1).

2.3 Antioxidant properties

Uric acid is known to possess antioxidant properties, and is believed to be one of the most abundant sources of antioxidant activity in human plasma. It is capable of

scavenging powerful free radicals including superoxide, hydroxyl radical, and singlet oxygen, and can chelate transition metals, which promote free radical liberation. Its antioxidant properties have been identified both *in vitro* and *ex vivo*. The importance of uric acid as a physiological antioxidant has been underpinned by studies that demonstrate it contributes more than two-thirds of all free radical scavenging capacity of human serum [73, 118].

2.3.1 Uric acid and lipid peroxidation

In addition to its role as a free radical scavenger, uric acid is known to interact directly with peroxynitrite, which may have additional effects on human cardiovascular redox conditions. Peroxynitrite is a powerful radical that is capable of damaging phospholipid components of endothelial cell membranes. Lipoproteins are particularly vulnerable to attack by free radicals at sites of polyunsaturated fatty acid carbon double bonds, and the interaction with peroxynitrite in the presence of oxygen causes rapid formation of peroxyl radicals. In turn, these react with fatty acids to generate lipid peroxides, which are unstable radicals capable of propagating redox chain reactions. The interaction of lipid peroxides and cellular components, so called lipid peroxidation, results in formation of a number of toxic secondary oxidation products, which can cause further direct tissue damage. Their quantification in bodily fluids, for example plasma or urinary isoprostane concentrations, is indicative of the extent of local lipid peroxidation and provides a biological marker of oxidant activity *in vivo* [119].

Uric acid is particularly effective in quenching hydroxyl, superoxide and peroxynitrite radicals, and may serve a protective physiological role by preventing lipid peroxidation [120]. Uric acid concentrations have been found to increase during acute oxidative stress and ischaemia in a number of organs and vascular beds. The consequent increase in antioxidant defences conferred by high uric acid concentrations has been proposed as a compensatory mechanism that protects against increased free radical activity [121]. Animal models have shown that, for example, transient middle cerebral artery occlusion causes a significant increase in cerebral uric acid concentrations, which can remain elevated for several days after the

primary ischaemic insult [122]. Physiological concentrations of uric acid protect hippocampal neurons *in vitro* against excitotoxic and metabolic injury during acute ischaemia. Raising circulating uric concentrations *in vivo*, by means of direct administration, reduced infarct volume and improved functional outcome in a rat model of acute ischaemic stroke [123]. This suggests that high concentrations of uric acid protect against oxidative tissue damage during acute neuronal ischaemia.

2.3.2 Uric acid and peroxynitrite

Nitric oxide is normally subjected to rapid degradation, which is increased in the presence of excess peroxynitrite. Peroxynitrite is a potent oxidant that is capable of causing endothelial injury by direct interaction, so that in the setting of oxidative stress, the increased peroxynitrite concentrations might be an important mechanism underlying endothelial dysfunction. Uric acid, as a powerful antioxidant, is capable of quenching peroxynitrite activity. Animal models of cardiac reperfusion injury and cerebral ischaemia have shown that uric acid protects against peroxynitrite-mediated protein nitration, thereby conserving tissue viability [124]. Furthermore, uric acid has been found to react with peroxynitrite to form a stable nitric oxide donor [125] and, therefore, high uric acid concentrations might conserve nitric oxide dependent vascular function in the setting of oxidative stress.

2.3.3 Uric acid and superoxide dismutase

Superoxide dismutase normally acts to convert superoxide free radicals to less potent hydrogen peroxide radicals, thereby preventing superoxide-mediated nitric oxide degradation. As a consequence, superoxide dismutase is critical in maintaining endothelial and vascular integrity. Furthermore, superoxide dismutase is itself degraded by hydrogen peroxide [126]. Uric acid is capable of preventing hydrogen peroxide-mediated degradation of superoxide dismutase, accompanied by the formation of an intermediary urate radical [126]. While potentially a pro-oxidant, the urate radical is itself significantly less active than classical oxidants, and is rapidly regenerated back to uric acid in the presence of ascorbic acid [126]. In this manner, uric acid appears to serve a role in preserving superoxide dismutase activity, and attenuating hydrogen peroxide activity.

2.4 Evolutionary aspects of uric acid metabolism

Uric acid is metabolised to allantoin by urate oxidase in virtually all other mammalian species apart from humans and higher apes. In hominoids, the gene encoding urate oxidase has been identified on chromosome 1p22, but is not expressed due to two non-sense mutations at codon positions 33 and 187, and an aberrant splice site [128, 129]. As a consequence, circulating uric acid concentrations are significantly higher in hominoids than most other species. The pattern of genetic mutations that prevent expression of urate oxidase, and other uric acid-degrading enzymes (allantoinase, allantoinicase, ureidoglycolate lyase and urease), has suggested that loss of urate oxidase has arisen through selective pressure [130]. This has generated speculation over the potential evolutionary benefits that may be conferred to hominoids by high serum uric acid concentrations. For example, it has been suggested that the antioxidant properties of uric acid might serve to defend against the oxidants, to which exposure cumulatively increases with longevity [131]. A positive correlation has been observed between mean serum uric acid concentrations and life span across a wide range of mammalian species [132].

The time frame of the urate oxidase gene mutations is estimated to have occurred between 8 and 24 million years ago, in the Miocene era. During this early evolutionary period, the hominoid diet is likely to have consisted primarily of fruit and vegetables, providing a daily sodium intake of less than 700 mg (1.9 g sodium chloride). Coupled with arid climatic conditions prevailing at that time, early hominoids are likely to have had greater dependence on salt-retaining mechanisms for blood pressure homeostasis, such as the renin-angiotensin-aldosterone system. Animal models have shown that in the setting of a low salt diet, uric acid causes increased blood pressure by enhancing sodium sensitivity and activation of the renin-angiotensin-aldosterone system [133]. Uric acid causes morphological renal vascular changes so that increased salt sensitivity persists, even after restoration of normal uric acid concentrations [134]. The effect of high circulating uric acid concentrations on salt sensitivity has not been studied in humans. It is tempting to speculate that higher concentrations might have provided an evolutionary advantage to early hominoids, by means of conserving blood pressure in the setting of low salt intake,

whereas. However, it is possible that this effect might be unfavourable in the presence of an adequate or high salt intake.

Chapter 3.

Uric acid and cardiovascular disease

3.1 Epidemiological associations with cardiovascular risk

Observational studies show that serum uric acid concentrations are higher in patients with established coronary heart disease compared to healthy controls [135]. Furthermore, strong epidemiological links between elevated serum uric acid concentration and increased cardiovascular risk have been recognised for many years [101, 136]. However, hyperuricaemia is associated with a number of confounding factors, many of which recognised as independent cardiovascular risk factors [137-142].

Table 4. Recognised associations of hyperuricaemia

Impaired renal function
Hypertension
Diuretic treatment
Increased waist-hip ratio & body mass index
Hypertriglyceridaemia
Hypercholesterolaemia
Impaired glucose tolerance
Hyperhomocysteinaemia
High C-reactive protein concentrations

These confounding factors cause difficulty interpreting relationships between serum uric acid concentration and cardiovascular risk in epidemiological studies. Many authorities have attempted to address the issue of confounding factors by the use of multivariate analyses. However, these do not fully exclude the possibility of residual confounding within such closely associated variables. Epidemiological studies in both unselected populations and high risk patient groups and have given conflicting results (see Tables 5 and 6). It appears that epidemiological data alone will not have sufficient power to discriminate whether high serum uric acid concentrations are independently related to increased cardiovascular risk, or indirectly linked through associations with other established risk factors.

Table 5. Epidemiological associations between serum uric acid concentrations and cardiovascular risk in unselected populations.

Study	Year	Ref.	Number	Duration (y)	Univariate correlation	Independent correlation
Framingham	1985	[144]	5209	26	Y	N
	1987	[145]	5127	30	Y	Women
	1999	[146]	6763	17.3	Women	N
Honolulu Heart	1975	[147]	8006	2	Y	Y
Study (men)	1995	[148]	2710	20	Y	Y
	1996	[149]	6411*	21	Y	Y
Chicago Heart	1979	[150]	24997	5	Y	Y
Assoc. (women)	1989	[151]	6797	11.5	Y	Y
NHANES I	1995	[152]	5421	13.5	Y	Women
	2000	[153]	5926	16.4	Y	Y
ARIC study	2000	[154]	13504	8	Women	N
British Regional	1997	[155]	7688	16.8	Y	N
Heart Study			men			
Social Institution	1982	[156]	6355	5	Y	N
of Finland						
Gothenburg**	1988	[157]	1462	12	Y	Y
			women			
MONICA	1999	[158]	1044	8	Y	Y
project**			men			
Evans County	1973	[159]	2212	-	Y	N
Study***						
NHANES III***	1999	[160]	16025	-	Y	Y

*alcohol abstainers, **all-cause mortality, ***prevalence

Table 6. Epidemiological associations between serum uric acid concentrations and cardiovascular risk in high risk groups.

Study	Year	Ref.	Number	Duration (y)	Univariate correlation	Independent correlation
Elderly Patients:						
CASTEL study*	1993	[161]	318 (> 80 y)	7	Y	Y
McArthur Studies	2001	[162]	870 (>65 y)	7	N	N
Patients with hypertension:						
HDFPC research	1985	[163]	10940	5	Y	Y
group	1987	[164]	3693**	5	Y	Women
Work site study	1999	[165]	7978	6.6	Y	Y
PIUMA study	2000	[166]	1720	4	Y	Y
European Working Party	1991	[167]	822	3	Y	N
SHEP study	2001	[168]	4327	5	Y	Y
Syst-China	2001	[169]	1873	3	Y	Y
Syst-Eur	2002	[170]	4522	2	N	N
Patients with established coronary artery disease:						
Coronary Drug Project	1976	[171]	2789	3	Y	N
Atherogene Study	2002	[172]	1017	2.2	Y	Y
French Canadian Study***	1973	[173]	1002	-	N	N
Spokane study***	2001	[142]	277	-	Y	Women
Patients with type 2 diabetes:						
Lehto et al.	1998	[174]	1017	7	Y	Y

*all-cause mortality, **thiazide treated subgroup, ***coronary artery disease severity

3.2 Associations between uric acid and cardiovascular risk

3.2.1 Uric acid and renal function

Renal clearance is the major route of uric acid elimination, and renal impairment is characteristically associated with increased serum uric acid concentrations. Furthermore, low renal blood flow stimulates tubular reabsorption of uric acid [141]. Around 25% to 40% of patients with a clinical history of gout have impaired renal function, with evidence of histologic injury in the majority [175]. Typically, the renal lesions seen in patients with gout consist of variable degrees of arteriolosclerosis, glomerulosclerosis, and interstitial fibrosis, often with focal deposition of urate crystals in the outer medulla. Many authorities have ascribed the renal injury to coexistent hypertension or aging-associated renal disease [176]. However, these factors alone might not entirely account for all of the renal injury observed [177]. Lowering serum uric acid concentrations in patients with a clinical history of gout has been found to improve renal function in some studies [178, 179], but not all [180].

A number of clinical studies have shown that high serum uric acid concentrations are an important risk marker for renal impairment across unselected adult populations [176, 181]. High uric acid concentrations appear to contribute to progression of renal impairment, as found in one study [182], but this has been refuted by the results of another study [183]. In patients with IgA nephropathy, uric acid concentrations have been shown to be an independent predictor of deteriorating renal function [184, 185]. In patients with type 2 diabetes, hyperuricaemia confers a significantly greater risk of developing renal impairment [186]. However, in patients with accelerated hypertension, a close correlation has been found between baseline serum uric acid and creatinine concentrations, but high uric acid measurements did not predict subsequent decline in renal function [187].

On the basis of existing literature, it is unclear whether the known association between serum uric acid concentrations and renal impairment can be fully explained by reduced clearance alone. During exposure to extreme high serum uric acid values, for example those encountered in the comparatively rare setting of acute tumour lysis

syndrome, there is grossly increased uric acid excretion that may be accompanied by tubular and ureteric crystal formation (see Section 3.5.2). It is not known whether chronic exposure to high circulating concentrations are causally associated with renal injury in wider populations.

3.2.2 Uric acid and hypertension

Oxonic acid inhibits urate oxidase activity and thereby increases serum uric acid concentrations in animal models of hyperuricaemia. Dietary supplementation with 2% oxonic acid in rats has been shown to significantly increase blood pressure from 125 ± 15 to 143 ± 13 mmHg (mean ± SEM) at 4 weeks, and a close correlation was found between the resultant uric acid concentration and rise in blood pressure [133]. The effect persisted until death or euthanasia at 7 weeks, and was prevented by concurrent administration of allopurinol and a uricosuric agent [133]. A characteristic form of microscopic kidney injury and increased renin concentrations were noted, suggest a possible renal mechanism for the observed increase in blood pressure. Oxonic acid supplementation to Sprague-Dawley rats fed a low salt diet caused high blood pressure at 5 weeks, 143 ± 4 versus 126 ± 2 mmHg (mean ± SEM) in controls [188]. Histology and immunostaining showed pre-glomerular arteriolar hypertrophy, suggesting a renal mechanism. Blood pressure elevation and renal damage were prevented by allopurinol administration [188]. Findings from these animal models have tempted speculation that high serum uric acid concentrations might play a causal role in the development of hypertension in humans.

Renal function is a major determinant of serum urate concentration, and around one quarter of hypertensive patients have co-existent hyperuricaemia due to impaired renal uric acid clearance [189].

Asymptomatic hyperuricaemia has been found to predict future development of hypertension in unselected populations [190-194].

The US National Health and Nutrition Survey (NHANES) III showed that age-adjusted rates of myocardial infarction and stroke are higher across increasing serum

uric acid quartiles in male and female hypertensive patients [160]. In essential hypertension, high serum uric acid concentrations are associated with significantly increased cardiovascular risk, independent of renal function and other confounding factors. During a mean 6.6-year follow-up, the proportional hazard ratio associated with one standard deviation increase in serum uric acid concentration (29.2 μmol/l) was 1.22 (95% confidence interval 1.11 - 1.35), and higher than that found for comparable increases in blood glucose, cholesterol or systolic blood pressure [165]. Similarly, a significant association has been found between serum uric acid concentrations and cardiovascular and total mortality in patients with isolated systolic hypertension, after adjusting for renal function [169]. An association between serum uric acid concentration and pulse pressure has been identified in unselected populations, suggesting a possible mechanistic link between hyperuricaemia and isolated systolic hypertension [195].

Thiazide diuretics confer unequivocal benefits in patients with hypertension, and cause a significant reduction in cardiovascular and all-cause mortality [196]. Persistence of the relationship between elevated serum uric acid concentration and increased cardiovascular risk among thiazide treated patients has prompted speculation that uric acid elevation may attenuate some of their potential benefits [197]. An association has been found between high serum uric acid concentration and poorer response to anti-hypertensive therapy [198]. Despite this, a large recent clinical trial has reported that thiazide treatment reduces cardiovascular risk to a similar or greater extent than other anti-hypertensive agents associated with comparable reductions in blood pressure [199]. Interestingly, these findings have prompted speculation that the increase in serum uric acid concentrations associated with thiazide diuretics might be a mechanism by which treatment reduces cardiovascular risk [200], although this hypothesis has not been examined directly.

3.2.3 Uric acid and insulin resistance

Serum uric acid concentrations are closely associated with serum triglyceride and cholesterol concentrations, blood glucose, fasting and post-carbohydrate plasma insulin concentrations, waist-hip ratio and body mass index [101, 137-140]. Uric acid measurements are higher in subjects with impaired glucose tolerance than healthy subjects, and independently associated with components of the metabolic syndrome. Uric acid values are more closely associated with body mass index, blood pressure and glycaemia after oral glucose administration, than insulin sensitivity *per se* [201]. Among apparently healthy people, high serum uric acid concentrations have been found to predict weight gain, [193], impaired glucose tolerance and type 2 diabetes [192]. Elevated serum uric acid concentrations predict the subsequent development of diabetes mellitus, even in the presence of normal creatinine clearance and plasma glucose concentrations [202].

Insulin has a physiological renal tubular effect that reduces sodium and uric acid clearance [203]. Insulin resistance and type 2 diabetes are characterised by impaired insulin-mediated glucose metabolism in peripheral tissues, whereas renal sensitivity to insulin persists [203, 204]. This suggests that high serum uric acid concentrations might be secondary to hyperinsulinaemia, which is a characteristic finding in insulin resistance syndromes and type 2 diabetes. Insulin resistance is an independent predictor of coronary heart disease and stroke in apparently healthy adults [205]. Therefore, a non-causal relationship between high serum uric acid concentrations and cardiovascular risk might be explained by its role as an early marker of peripheral insulin resistance.

In patients with established type 2 diabetes, high serum uric acid concentrations predict stroke risk, independent of other identifiable risk factors [174], as described also in unselected patient populations [206]. Paradoxically, high serum uric acid concentrations in the setting of acute ischaemic stroke are associated with better clinical outcome [207]. This raises the possibility that uric acid, through a number of potential mechanisms, might play distinct roles in the development of atherosclerosis and its late ischaemic disease manifestations.

3.2.4 Uric acid and xanthine oxidase

The xanthine oxidoreductase gene encodes xanthine dehydrogenase, a 150 kDa protein. Xanthine dehydrogenase is constitutively expressed and catalyses the metabolism of hypoxanthine and xanthine to uric acid. Xanthine dehydrogenase requires NAD+ as an electron acceptor, thereby generating a stable reaction product, NADH. However, in the setting of oxidative stress, xanthine dehydrogenase is converted to xanthine oxidase by thiol oxidation of sulphhydryl residues, or irreversible proteolytic cleavage [208]. Unlike xanthine dehydrogenase, xanthine oxidase is unable to utilise NAD+ as an electron acceptor and, instead, reduces molecular oxygen to the highly reactive superoxide radical [209]. Xanthine oxidase-derived reactive oxygen species have been implicated in a number of pathologies, including myocardial ischaemia, cerebral ischaemia and tissue reperfusion injury.

Under conditions of tissue ischaemia, local uric acid concentrations become significantly increased [210]. This is thought to result from enhanced availability of purine substrate, due to increased local formation and release of adenosine [211], alongside up-regulation of xanthine oxidase activity [212]. For example, cardiac ischaemia caused by transient coronary artery occlusion is associated with acute increases in local uric acid concentrations [213]. Tourniquet-induced lower limb exsanguination causes a 5-fold increase in systemic vascular xanthine oxidase activity during reperfusion, and serum uric acid concentrations become significantly elevated for at least 2 hours [214].

To date, no specific xanthine oxidase inhibitor is available. Allopurinol, and its active metabolite oxypurinol have been widely used in a therapeutic setting, and in clinical research as a means of inhibiting xanthine oxidase. However, both compounds bind at a common molybdenopterin cofactor site so as to inhibit the activity of both xanthine dehydrogenase and xanthine oxidase. Inhibition of xanthine oxidase reduces formation of superoxide radicals, and has been shown to reduce oxidative stress in situations where activity of this enzyme is significantly increased, for example congestive heart failure [215]. However, inhibition of both xanthine dehydrogenase and xanthine oxidase causes a significant reduction in uric acid

concentrations. Uric acid has been shown to protect against free radical mediated injury in the setting of ischaemia. Loss of antioxidant capacity, as a consequence of allopurinol-mediated reduction in uric acid concentrations exacerbates oxidative stress in certain situations [216, 217]. Therefore, effects of allopurinol and oxypurinol on vascular redox state and oxidative stress are likely to depend on the extent to which xanthine oxidase is active. High serum uric acid concentrations appear to offer an important marker of enhanced vascular xanthine oxidase activity, associated with sub-clinical tissue ischaemia. This is consistent with an inverse relation between serum uric acid concentrations and lower limb blood flow found in patients with chronic heart failure. This mechanism offers a potential explanation for a non-causal association between high serum uric acid concentrations and conditions associated with increased cardiovascular risk.

Endothelial cells of microvascular origin are rich in xanthine dehydrogenase, and uric acid predominates as the final product of local purine metabolism. Conversely, xanthine dehydrogenase is relatively lacking in endothelial cells associated with medium and large arteries, and hypoxanthine is the predominant final product of purine metabolism [218]. This interesting observation suggests that the comparative lack of uric acid availability within large vessel walls, and resulting loss of antioxidant defences might contribute to the preponderance of atherosclerosis development at these sites, although this remains unproven.

3.2.4.1 Xanthine oxidase and cardiovascular risk

Vascular xanthine oxidase activity is significantly up-regulated in both experimental and human congestive heart failure [219, 220]. Serum uric acid concentration is a particularly powerful marker of increased cardiovascular risk in such patients, and the relative risk ratios associated with concentrations 400-600, 600-800 and >800 μmol/l are 1.8, 6.3 and 18.5 respectively, compared to those < 400 μmol/l [221]. Uric acid remains an important prognostic indicator, even when the potential confounding effects of renal function and diuretic use are taken into consideration [222]. Allopurinol has been found to significantly improve serum antioxidant capacity and

endothelial function in patients with chronic heart failure [215, 223], underpinning the importance of excess xanthine oxidase activity in this group.

Excess xanthine oxidase activity also appears to play a significant role in mediating oxidative stress in patients with diabetes mellitus, and administration of allopurinol affords protection against free radical mediated vascular injury in this patient group [224]. Furthermore, allopurinol has been shown to allow restoration of endothelial function in patients with type 2 diabetes mellitus, associated with increased serum antioxidant capacity [225]. The contribution of increased xanthine oxidase activity to vascular dysfunction is less certain in the presence of other major cardiovascular risk factors. In patients with hypertension and hypercholesterolaemia, there is clear evidence that excess superoxide participates in vascular dysfunction [226]. Animal and human studies are inconsistent with regard to xanthine oxidase as an important source of superoxide radicals [227-229], whereas NAD(P)H oxidase or endothelial nitric oxide synthase may be more important sources in these patients [230].

3.2.5 Uric acid and alcohol intake

A significant j-curve relationship has been identified between alcohol intake and serum uric acid concentrations [231], and a similar relationship has been shown between alcohol ingestion and cardiovascular risk [232]. These findings indicate that alcohol intake is an important confounding factor in examining relationships between serum uric acid concentrations and cardiovascular risk. Alcohol ingestion can cause with increased serum uric acid concentrations through a number of mechanisms: (1) high purine content of some alcoholic beverages, (2) increased adenine nucleotide turnover [233], and (3) raised lactate concentrations that decrease renal tubular secretion of uric acid by competing for the organic anion transporter [115]. Despite alcohol intake being an important confounding factor, an independent relationship between serum uric acid concentration and cardiovascular risk has been shown in middle-aged alcohol-abstinent men included in the Honolulu Heart Study [147].

3.3 Potential pathophysiological links between uric acid and cardiovascular risk

3.3.1 Pro-oxidant effects of uric acid

Despite its widely acknowledged antioxidant properties, like other antioxidants uric acid may exert pro-oxidant effects under certain circumstances. For example, uric acid has been found to promote oxidation of low density lipoprotein *in vitro* [234, 235]. Oxidation of low density lipoprotein is believed to be an important step in the development and progression of atherosclerosis, and high uric acid concentrations might significantly impair vascular function if such a mechanism operates *in vivo*. Its pro-oxidant properties have been found to be inhibited by vitamin C [236], underpinning the importance of functional interactions between various aqueous anti-oxidants.

3.3.2 Uric acid and vascular inflammation

Uric acid is able to traverse dysfunctional endothelial cells, and accumulate in crystals nested within developing atherosclerotic plaques [237]. Monosodium urate crystals have been shown to stimulate granulocyte adherence to the endothelium, and liberation of peroxide and superoxide free radicals [238, 239]. Therefore, uric acid appears capable of promoting atherosclerosis progression by accumulating at sites of endothelial dysfunction, stimulating leukocyte activation and propagating endothelial dysfunction. It is likely that in the presence of endothelial dysfunction, high serum uric acid concentrations will confer a greater predisposition for sub-endothelial uric acid accumulation. Qualitative and quantitative analyses of the protein adsorptive properties of monosodium urate monohydrate crystals have shown that they preferentially adsorb immunoglobulin G from normal human serum, to which it binds with high affinity [240]. This property suggests a means of Fc receptor mediated sub-endothelial phagocytosis, which might further promote localised vascular inflammation.

Uric acid has been shown to exert a dose-dependent mitogenic effect on rat aortic vascular smooth muscle *in vitro* [241]. Half-maximal induction of vascular smooth muscle DNA synthesis occurred at 150 μmol/l and maximal effects at 250 μmol/l.

While both concentrations are considerably in excess of serum concentrations normally found in the rat, they are similar to those to which humans are ordinarily exposed (95% reference range 120 - 420 μmol/l). The mitogenic effect was specific for vascular smooth muscle, not observed in response to other purine metabolites or aqueous antioxidants [241]. This observation indicates that uric acid is capable of exerting a direct effect on vascular smooth muscle, and suggests a further possible mechanism by which sub-endothelial uric acid crystal accumulation might contribute to the progression of atherosclerosis.

A consistent relationship has been noted between elevated serum uric acid concentration and circulating inflammatory markers in animal models and humans [242-244].

3.3.3 Uric acid and enhanced platelet aggregability

For more than forty years, enhanced platelet aggregability has been proposed as a potential biological mechanism of increased cardiovascular risk in the setting of high serum uric acid concentrations [171, 245, 246]. Monosodium urate crystals have been found to promote the release of platelet components *in vitro* [247]. Furthermore, the observation of high serum uric acid concentrations in patients with thromboangitis obliterans lent support to the possibility that uric acid might influence platelet activation *in vivo* [248]. Patients with a clinical history of gout have been shown to exhibit characteristic abnormalities of platelet morphology, which are restored by treatment with allopurinol [249]. Furthermore, treatment with sulphinpyrazone, a uricosuric agent, has been shown to reduce platelet aggregability and improve clinical outcome in patients who had suffered a recent myocardial infarction [250]. However, interpretation of these studies is difficult because both allopurinol and sulphinpyrazone are highly protein-bound, and capable of reducing platelet aggregability independent of effects on serum uric acid concentration.

Tranylcypromine (a prostacyclin synthetase inhibitor) catalyses ADP-induced platelet aggregation. However, tolerance to this effect develops rapidly due to inactivation of cyclooxygenase free radicals [251]. Uric acid has been shown to

prevent tolerance and prolong platelet activation, which was thought due to its antioxidant properties [251]. High uric acid concentrations might be capable of promoting platelet aggregation if a similar mechanism were operating *in vivo*, although this has not yet been demonstrated.

Rats treated with dietary oxonic acid supplementation, to increase serum uric acid concentrations have been shown to exhibit increased ADP-induced and thrombin-induced platelet aggregation, which is restored after normalisation of uric acid concentrations [252].

A study in 12 patients with a clinical history of gout found normal platelet aggregation and adhesion, which were not influenced by the uric acid level [253]. However, this study is likely to be confounded by wide variability in the baseline characteristics of this group. In another clinical study, ADP, adrenaline and collagen mediated platelet aggregation were studied in 5 healthy subjects. Treatment with ribonucleic acid 3 g/day and allopurinol, to raise and lower serum uric acid concentrations respectively, had no effect on platelet aggregability [254], although the evoked changes in serum uric acid concentrations were comparatively small.

Halofenate has lipid-lowering and uric acid-lowering properties *in vivo*. Administration of halofenate to 14 patients with type IV hyperlipoproteinemia and hyperuricaemia had no effects on platelet aggregability or blood coagulability [255], suggesting that lowering serum uric acid concentrations did not influence platelet function. However, because the intervention altered both lipid and uric acid concentrations, it is difficult to draw conclusions about the possible independent effects of each.

At present, the relationship between serum uric acid concentration and platelet aggregability is well understood. An association has been recognised between high serum uric acid concentrations and increased platelet aggregation, and a number of potential biological mechanisms have been proposed on the basis of laboratory and animal data. Clinical studies have been unable to identify a clear link between uric

acid and platelet function, however, these have involved small subject numbers and been limited by a number of potential confounding factors.

3.3.4 Uric acid and plasma viscosity

A recent study in men with borderline and established hypertension found that serum uric acid concentration tended to correlate with whole blood viscosity at low and high shear rates in both groups, but these findings were not statistically significant [256]. The potential mechanisms by which uric acid might influence plasma viscosity are unclear. Previous studies have demonstrated an inverse relationship between insulin resistance and increased blood viscosity [257, 258], and an association has been found between serum triglyceride concentrations and plasma viscosity in adults attending a cardiovascular prevention clinic [259]. Therefore, a relationship between high uric acid concentrations and increased viscosity might be explained by its confounding association with plasma triglycerides. The direct impact of high serum uric acid concentrations on plasma viscosity is not known, and its potential contribution to increased viscosity in insulin resistance syndromes has not previously been examined.

3.2.5 Uric acid and salt sensitivity

In certain people, exposure to high dietary sodium causes a significant increase in systemic blood pressure, for example more than a 10-20 mmHg increase in systolic blood pressure. Such individuals are described as having 'salt sensitivity', which may predispose to the development of hypertension. Animal models have demonstrated that high serum uric acid concentrations increase sodium sensitivity and activate the renin angiotensin aldosterone system, thereby increasing blood pressure (discussed in Section 2.4). If a similar mechanism operates in humans, then high serum uric acid concentrations might predispose to hypertension by increasing salt sensitivity in the context of a high dietary salt intake. One previous study showed that salt-sensitive individuals, defined by blood pressure response to dietary salt loading, had higher serum uric acid concentrations than healthy counterparts [260]. The relationship between hyperuricaemia and salt sensitivity offers a plausible mechanism to explain the observed associations between raised serum uric acid concentrations and high

blood pressure and cardiovascular risk. Clinical studies are required to identify whether such a mechanism operates in people.

3.4 Causal versus non-causal association with cardiovascular risk

The key criteria for causality proposed by Bradford Hill are valuable in assessing the significance of epidemiological observations [261].

Table 7. Bradford Hill criteria applied to the relationship between serum uric acid concentrations and cardiovascular risk.

Criterion	Uric Acid and Cardiovascular Risk
Strength of the association	Most epidemiological studies show cardiovascular hazard ratio < 2 between lowest and highest categories
Dose-response relationship	Appears to be present in most studies, but often negated by consideration of confounding factors
Consistency	Consistent association with risk in many studies, particularly for women and less so for men
Specificity	Existing findings are non-specific due to associations between uric acid and a variety of confounders
Temporal relation	Reasonable temporal relationship, and high concentrations predict future events in a number of subject groups
Biological plausibility	A number of potential mechanisms proposed, but none proven to be relevant

On the basis of these criteria, a causal relationship between uric acid and cardiovascular disease appears possible, albeit somewhat weak. In order to address the role of uric acid as a potential causal risk factor, basic clinical research needs to identify possible biological mechanisms by which high concentrations might impair vascular function. This thesis specifically examines the effects of raising or lowering serum uric acid concentrations on mechanisms linked to atherosclerosis development such as endothelial function, large arterial stiffness, baroreflex sensitivity and circulating antioxidant activity, in order to establish biologically plausible mechanistic links. Additional longitudinal studies are needed to address the impact of manipulating serum uric acid concentrations on long-term cardiovascular risk.

3.5 Uric acid and other diseases

3.5.1 Acute Gout

Gout is a clinical disorder characterised by acute joint inflammation, usually at a single medium to large joint. It is predominantly an inflammatory disorder, and appears to be triggered by acute changes in the composition of pre-formed uric acid crystals. The precise mechanisms involved are unclear, but certain factors appear capable of inducing instability within uric acid crystals, which triggers an ensuing localised inflammatory cascade. Physiochemical instability of uric acid crystals can result from local changes such as reduced temperature, increased or decreased local pH, increased calcium concentrations, and increases or decreases in circulating uric acid concentration.

In the setting of eating a purine-free diet, the total body pool of exchangeable uric acid is estimated at 1.2 g in men and 0.6 g in women. By contrast, patients prone to acute gout or uric acid deposition in soft tissues appear to have substantially increased total body urate reservoirs, up to 18-30 g [116]. It is unclear why certain individuals might accumulate abnormally high amounts of uric acid, thereby predisposing to gout. Uric acid crystals accumulate throughout life and, given that the existence of formed uric acid crystals is a prerequisite for developing acute gout, it is unsurprising that its occurrence is rare in youth and becomes more prevalent with advancing age.

Serum uric acid concentrations are a comparatively unreliable indicator of the risk of developing acute gout. For example, in individuals with severe chronic hyperuricaemia and serum uric acid concentrations exceeding 540 μmol/l, the annual incidence of gouty arthritis is less than 5% [262]. By contrast, the vast majority of patients who develop acute gout have serum uric acid concentrations within the normal reference range. Most authorities advocate that the risks of developing acute gout are more closely related to high total body uric acid content and chronic uric acid accumulation, rather than to short term increases in serum concentrations.

3.5.2 Uric acid nephropathy

Hyperuricaemic nephropathy can be considered as two distinct entities. Firstly, 'classical' chronic uric acid nephropathy is associated with high serum uric acid concentrations and clinical history of gout. The condition is characterised by precipitation of uric acid microcrystals within the renal interstitium, which evokes a localised inflammatory response. A peripheral rim of leukocytes and fibrosis surrounds a central monosodium urate crystal, which gives the hallmark histological appearance of a renal tophus, and is often accompanied by decreased glomerular filtration rate. In an analysis of more than 11000 autopsy cases, chronic hyperuricaemic nephropathy was identified in only 0.3% of cases, which is substantially fewer than predicted on the basis of the prevalence of hyperuricaemia [263]. Furthermore, renal impairment can be attributed to alternative causes in the vast majority of such cases [263, 264]. The presence of renal tophi is not dependent on high urinary uric acid concentrations [263]. Currently, the relationship between chronic hyperuricaemia and renal impairment remains poorly understood, and it remains uncertain if chronic renal impairment precedes the formation of renal tophi or *vice versa*.

By way of contrast, acute uric acid nephropathy is associated with sudden, and often striking increases in serum uric acid concentration. These patients may be younger, and there is rarely any clinical history of gout. The majority of cases of acute uric acid nephropathy occur in patients with underlying haematological malignancy, or those receiving cytotoxic treatment, due to rapid cell turnover and overwhelming urate production [264, 265]. There is gross hyperuricosuria, associated with uric acid precipitation in tubules and collecting ducts, which can result in obstructive nephropathy and acute renal impairment [110, 266]. With appropriate prophylactic measures and treatment, including intravenous hydration and uric acid lowering treatment, renal function can fully recover.

Chapter 4.

Hypotheses and Aims

4.1 Uric acid as an independent cardiovascular risk factor

A key hypothesis underpinning the early studies outlined in this thesis was that uric acid might be an independent cardiovascular risk factor, in view of the established associations between high serum concentrations, vascular dysfunction and increased cardiovascular risk. An extension of this hypothesis was that elevated serum uric acid concentrations might mediate increased risk through mechanisms common to other established independent cardiovascular risk factors.

The aim of the initial studies was to develop a suitable vehicle for localised and systemic administration of uric acid, so as to allow the effects of high serum concentrations to be examined. The effects of raising serum uric acid concentrations on endothelial function, endogenous fibrinolysis, large arterial compliance and baroreceptor reflex sensitivity *in vivo*, and plasma viscosity and platelet aggregability *ex vivo*, were examined in healthy subjects free of major cardiovascular risk factors.

4.2 Uric acid as an antioxidant

The existing evidence that uric acid exhibits free radical scavenging effects prompted further consideration of the potential importance of its antioxidant properties. In contrast to the rationale underlying the initial series of clinical studies, a secondary hypothesis was that uric acid might serve a protective role, as an antioxidant, and protect against vascular dysfunction in the setting of oxidative stress.

A series of experiments was designed to examine the impact of high uric acid concentrations on serum antioxidant capacity, and the consequent effects on vascular function in the setting of acute oxidative stress, evoked by aerobic physical exercise, and chronic oxidative stress, as found in regular smokers and patients with type 1 diabetes.

4.3 Uric acid as an independent risk factor in type-2 diabetes

An additional hypothesis was that high uric acid concentrations might make a specific contribution to cardiovascular risk in patients with type 2 diabetes, based on particularly strong associations between type 2 diabetes, hyperuricaemia and

endothelial dysfunction. An extension of this hypothesis was that lowering serum uric acid concentrations might improve vascular function in this patient group.

An additional series of clinical studies was designed to evaluate the effects of systemic urate oxidase administration, as a means of lowering serum uric acid concentration, on endothelial function and large arterial stiffness in patients with type 2 diabetes.

Chapter 5.

Materials and Methods

5.1 General

5.1.1 Ethical considerations

For all studies described in this thesis, the relevant protocols were submitted to the Lothian Health Board local research ethics committee, Research and Development department of the Lothian University Hospitals NHS Trust, and Health and Safety department of the University of Edinburgh for consideration. Studies were undertaken only after appropriate approval had been granted. The University of Edinburgh provided compensation arrangements where appropriate, and all studies were conducted in accordance with the appropriate Standard Operating Procedures in place within the Clinical Research Centre of the University of Edinburgh. In all cases, studies were performed in accordance with Good Clinical Practice, and in keeping with the principles outlined in the Declaration of Helsinki.

5.1.2 Subject recruitment

In all cases, subjects were provided with written 'Patient Information Sheets' as approved by the local research ethics committee, and given at least 24 h to consider these before deciding whether to participate. Subjects were recruited to participate in the study only if they were willing to provide informed consent, documented in writing, and if all inclusion criteria were met and there was no exclusion criterion.

5.1.2.1 Healthy subjects

Healthy volunteer subjects were recruited from a community database of healthy volunteers held in the Clinical Research Centre of the University of Edinburgh. Inclusion criteria were men or women, aged 18 to 45 y. Exclusion criteria were elevated blood pressure (> 160/100 mmHg), clinical history of joint, kidney or cardiovascular disease, taking any prescribed medication, taking any over the counter medications in the previous week, regular tobacco use, serum creatinine > 110 μmol/l, or serum uric acid > 420 μmol/l. For female subjects, an additional exclusion criterion was pregnancy. A human chorionic gonadotrophin (hCG)-based urinary pregnancy test was performed in all female subjects apart from those who had undergone previous hysterectomy or sterilisation more than 2 years before.

5.1.2.2 Regular smokers

Apparently healthy tobacco users were recruited from a community database held in the Clinical Research Centre of the University of Edinburgh. Inclusion criteria and exclusion criteria were identical to those of the healthy subject group, except for the following additional criteria. Inclusion criterion: smoking at least 10 cigarettes per day every day for at least 6 months. Exclusion criterion: unwilling or unable to abstain from smoking during the clinical observation phase of the study within the Clinical Research Centre.

5.1.2.3 Patients with type 1 diabetes mellitus

Patients with type 1 diabetes were identified from the outpatient diabetes clinic at the Western General Hospital. Subjects were provided with study documentation at the time of attendance at the clinic, which they could take away and consider. Inclusion and exclusion criteria were identical to those of the healthy subject group, except that the use of insulin was permitted. Patients with type 1 diabetes who required any other medications were excluded from participation, as for healthy subjects.

5.1.2.4 Patients with type 2 diabetes mellitus

Patients with type 2 diabetes were identified from the outpatient diabetes clinics at the Western General Hospital and Eastern General Hospitals. Subjects were provided with study documentation at the time of attendance at the clinic, which they could take away and consider. Inclusion and exclusion criteria were identical to those of the healthy subject group, except that the use of insulin and/or oral anti-hyperglycaemic medications were permitted. Patients with type 2 diabetes who required any other medications were excluded from participation, as for healthy subjects.

5.2 Drug preparation and administration

All drugs and solutions were stored in accordance with the manufacturers recommendations, and prepared on the day of administration using aseptic techniques and in accordance to local Standard Operating Procedures.

5.2.1 Uric acid and lithium carbonate

Uric acid and lithium carbonate were supplied in powder form with purity >99.99% (Ultrapure preparations, Sigma Chemical Company, Poole, UK). Each was reconstituted in sterile dextrose solution (Baxter Healthcare, Norfolk, UK), and filtered (0.22 μm Millex, Millipore, Molsheim, France) prior to administration to remove potential pyrogens. Laboratory analyses of filtered 4% dextrose vehicle/0.1% lithium carbonate containing uric acid was performed prior to administration to ensure that filtration had not lessened the quantities of uric acid in solution.

Table 8. Laboratory confirmation of lithium and uric acid concentrations in aqueous solutions used for study administration

Vehicle	**Assumed lithium concentrations:**				
	Li_2CO_3 (mmol/l) (F.W. = 73.89 g)	13.5			
	Free Li (mmol/l)	27.1			
	Actual measured lithium concentration:				
	Lithium assay (mmol/l)	27.5			
Uric acid	**Assumed uric acid concentrations:**				
	Uric acid (mg/ml)	0.5	1.0	2.0	4.0
	Uric acid (mmol/l) (F.W. = 168.1 g)	3.0	5.9	11.9	23.8
	Actual measured uric acid concentrations:				
	Uric acid assay (mmol/l)	3.0	5.7	16.2	27.2

5.2.2 Urate oxidase

As discussed in 2.4, urate oxidase is an enzyme that catalyses the metabolism of uric acid to more soluble waste products that are cleared by renal elimination. A pharmaceutical-grade preparation (Fasturec®, Sanofi-Synthelabo Pharmaceutical, Paris, France) was administered parenterally to lower circulating uric acid concentrations. Urate oxidase was supplied in anhydrous form, in 1.5 mg vials, and reconstituted in 5 ml sterile saline immediately prior to study administration.

5.2.3 Acetylcholine, sodium nitroprusside, bradykinin, L-NMMA

Acetylcholine (CIBAVision-Ophthalmics, Southampton, UK) and sodium nitroprusside (David Bull Laboratories, Warwick, UK) were supplied as pharmaceutical grade preparations. L-N^G-monomethylarginine and bradykinin (both Calbiochem-Novobiochem, Nottingham, UK) were supplied as synthetic compounds with greater than 99.99% purity determined by HPLC. All vasoactive drugs were diluted to the required concentrations in 0.9% sterile saline (Baxter Healthcare, Norfolk, UK) immediately before each study.

5.2.4 Other reagents

Lidocaine was used for local anaesthesia during venous and arterial cannulation procedures (1% Xylocaine®, AstraZeneca, Luton, UK).

5.3 Haemodynamic measurements

5.3.1 Heart rate and blood pressure

Heart rate and brachial blood pressure measurements were recorded in the dominant arm using a validated oscillometric device (HEM-705CP, Omron, Japan) [267].

5.3.2 Cardiac index and systemic vascular resistance index

Cardiac output was assessed non-invasively using transthoracic bioimpedance (NCCOM3-R7, BoMed, CA, USA) [268], and expressed as cardiac index (CI) to take account of inter-individual differences in body habitus that influence cardiac output. Cardiac index ($l.min^{-1}m^{-2}$)= cardiac output ÷ body surface area. Systemic vascular resistance index was calculated as mean arterial pressure ÷ divided by cardiac index. Where available, integrated mean arterial pressure was used in this calculation; in other cases, mean arterial pressure was calculated as diastolic pressure + ⅓(systolic-diastolic pressure).

5.4 Venous occlusion plethysmography

5.4.1 Blood flow measurement

Blood flow was measured in both forearms by venous occlusion plethysmography, as previously described [269, 270]. Forearm measurements were recorded using

mercury-in-silastic strain gauges connected to a microcomputer, and recorded using Chart® software (ADInstruments, Hastings, UK). Cuffs placed across both wrists were inflated to 200 mmHg during measurement, to exclude the hand circulation, and cuffs placed across the upper arms were inflated intermittently to 40 mmHg to occlude venous return from the forearm without impeding arterial inflow.

5.4.2 Brachial artery cannulation

The brachial artery of the non-dominant arm was cannulated using a 27-standard wire gauge steel needle (Cooper's Needle Works Ltd., Birmingham, UK) under local anesthesia, using an aseptic technique (Figure 2). Vasoactive drugs were administered via a 16-gauge epidural catheter (Portex Ltd., Kent, UK) connected to an IVAC P1000 syringe pump (Alaris Medical Ltd., Hampshire, UK). The rate of infusion was kept constant at 1 ml/min in all cases.

5.4.3 Determination of endothelial function

Intra-brachial saline was administered for at least 20 min to allow establishment of baseline blood flow. This was followed by administration of acetylcholine 7.5, 15 and 30 μg/min, sodium nitroprusside 2, 4 and 8 μg/min, and L-NMMA 2 and 4 mmol/min. The order of acetylcholine and sodium nitroprusside infusion was randomised between subjects, and constant between study visits. Drug infusions were separated by at least 20 min administration of saline to allow restoration of basal blood flow, and infused for 6 min at each dose. Measurements were taken during the last 3 min of each 6 min intra-brachial infusion, and the last five recordings averaged to determine flow in each arm. The ratio of blood flow in the infused versus non-infused limbs was considered, to account for any systemic effects, and responses to each vasoactive drug were expressed as a percentage change from baseline blood flow immediately preceding drug administration [269].

A

B

C

Figure 2. Brachial artery cannula insertion. (A) the course of the brachial artery is identified by palpation. (B) insertion of the 27-Gauge steel needle attached to a Portex catheter. (C) the needle is advanced carefully, observing for pulsatile blood flow in the catheter, which is flushed intermittently with saline to maintain patency.

5.5 Pulse waveform analysis

The dominant radial artery pulse waveform was assessed using applanation tonometry (SPC-301 micromanometer, Millar Instruments, USA), and a corresponding aortic pressure waveform was generated by PWA software (Sphygmocor, PWV, Australia) (Figure 3). Augmentation index is a validated measure of large arterial stiffness, calculated as the difference between the first and second central systolic blood pressure peaks, expressed as a percentage of pulse pressure [271]. Measurements were recorded in triplicate at each time-point, and the mean value used for data analysis.

Figure 3. Pulse waveform analysis using non-invasive applanation tonometry at the dominant radial artery site.

5.6 Baroreflex Sensitivity

Non-occlusive systolic blood pressure was measured continuously using a Portapres device (TNO, Amsterdam, Netherlands) with the photoplethysmographic cuff placed over the middle phalynx of the middle finger of the dominant hand. Continuous electrocardiogram signals were recorded simultaneously (Figure 4). Signals from both devices were recorded over a 15 min period, and analyzed offline using Chart

HRV software (ADInstruments, Hastings, UK). Baroreflex sensitivity was determined by two independent methods. Fast Fourier transformation of blood pressure and pulse interval data gave the total spectral power of the variability of each, and the formula $(power_{BP} / power_{PI})^{½}$ gave spontaneous baroreflex sensitivity by spectral analysis (BRS_{spec}). Parallel increases or decreases in blood pressure and pulse interval are representative of spontaneous baroreflex activity. Sequences of parallel increases or decreases over two or more consecutive beats were analysed, and the resulting slope (△pulse interval / △blood pressure) was used to represent baroreflex sensitivity by sequence analysis (BRS_{seq}).

The prognostic importance of baroreflex sensitivity had been ascertained by techniques that employed pharmacological manipulation of blood pressure [54]. Spectral analysis and sequence analysis techniques to measure spontaneous baroreceptor activity have been introduced more recently, and their relationship to clinical outcome is less well established. Despite this, spontaneous baroreflex sensitivity measurements by both methods have gained increasing acceptance. They are reproducible, and sensitive to orthostatic manoeuvres to activate or suppress baroreceptor activity [55, 56].

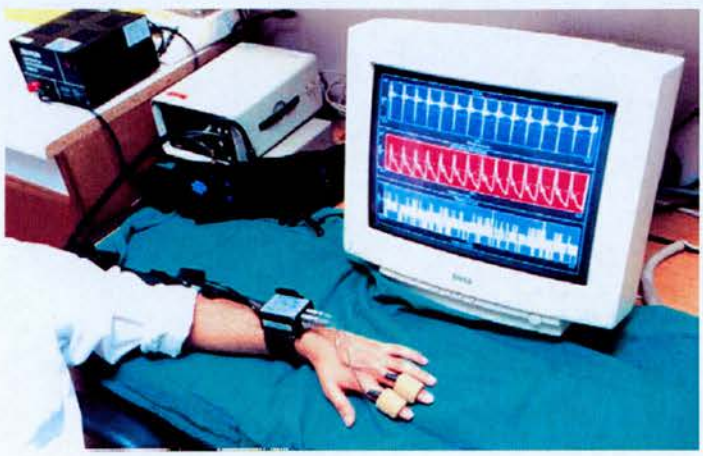


Figure 4. Spontaneous baroreflex sensitivity was determined by analysis of simultaneous ECG and systolic BP signals. Non-invasive continuous BP signals were generated using a Portapres device, which incorporated a non-occlusive photoplethysmographic cuff placed over the middle phalanx of the middle finger.

5.7 Assays

5.7.1 Uric acid

Serum uric acid concentrations were determined from 5 ml venous blood samples collected in serum gel tube (Sarstedt Ltd., Leicester, UK). Assays were performed in the Clinical Biochemistry department of the Western General Hospital, within 24 h of sample collection, using a colorimetric dry-slide method (Vitros, Ortho-Clinical Diagnostics, Amersham, UK). The standard reference range for this assay was 120-420 μmol/l, and intra-assay precision was 1.8%.

5.7.2 Antioxidant capacity

5.7.2.1 Enhanced chemiluminescence assay

Enhanced chemiluminescence (ECL) is based on the emission of light that occurs when luminol is oxidised by hydrogen peroxide, catalysed by horseradish peroxidase, and enhanced by phenol. Light emission is dependent on continuous production of free radicals, and is extinguished by biological fluids for a lag period that is proportional to their antioxidant content [272, 273]. A luminometer (Model 1251; BioOrbit, Turku, Finland) was used to establish the duration of the lag period caused by addition of serum. This was calibrated against a standard curve generated by addition of 0.16, 0.32, 0.48, and 0.64 μM Trolox, a water-soluble tocopherol analogue (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid, Sigma, Dorset, UK), and serum antioxidant capacity was expressed as μM of Trolox equivalents.

5.7.2.2 'Total Antioxidant Status' assay

The Total Antioxidant Status (TAS) assay (Randox Laboratories, Crumlin, UK) is based on the activity of the free radical ferrylmyoglobin, which is formed by oxidation of metmyoglobin by hydrogen peroxide. Ferrylmyoglobin interacts with the chromogen ABTS (2,2'-amino-di-[3-ethylbenzthiazole sulfonate]) to form a blue-green chromophore radical (ABTS·), which has maximal absorbance at 417, 645, 734, and 815 nm. Addition of serum causes a reduction in ABTS· radical activity that is proportional to its antioxidant content [274, 275]. ABTS· absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, East Sussex, UK), and

measurements were calibrated against a Trolox standard curve. Serum antioxidant capacity was expressed as μM Trolox equivalents.

5.7.3 8-isoprostaglandin $F_{2\alpha}$

Solid-phase extraction was performed using an Isosolute C18(EC) 100 mg/ 3 ml silica sorbent column (International Sorbent Technology, Mid Glamorgan, UK), and 8-*iso*-$PGF_{2\alpha}$ was eluted with ethylacetate: methanol (99: 1) buffer. Assays were performed on undiluted samples and after 1: 2 dilution with buffer, and each concentration was assayed in duplicate. 8-*iso*-$PGF_{2\alpha}$ concentrations were measured using an enzyme-linked immunosorbent assay (EIA) (Cayman Chemical Co., Ann Arbor, USA). The assay is based on competition between 8-*iso*-$PGF_{2\alpha}$ and acetylcholinesterase-8-*iso*-$PGF_{2\alpha}$ conjugate for limited amounts of 8-*iso*-$PGF_{2\alpha}$-specific rabbit immunoglobulin-G bound to 96-well plates. Ellman's reagent contains an acetylcholinesterase substrate, which formed a distinctive yellow reaction product whose absorbance was detected at 412 nm using a Vmax kinetic microplate spectrophotometer (Molecular Devices Ltd., Winnersh, UK). The extent of absorbance is proportional to the amount of conjugate in each well, which is inversely proportional to free 8-*iso*-$PGF_{2\alpha}$ concentrations during incubation [276]. Mean values were expressed as a percentage of maximal binding absorbance, and 8-*iso*-$PGF_{2\alpha}$ content was determined by comparison to absorbance of standard isoprostane concentrations (3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 pg/ml). The standard reference range was 4.6 - 65.9 pg/ml, and intra-assay precision was 6.0%.

5.7.4 Lithium

Serum lithium concentrations were determined by a standard reference laboratory method, using an automated colorimetric assay (Vitros dry slide, Johnson & Johnson, CA, USA).

5.7.5 Tissue plasminogen activator

Tissue plasminogen activator antigen concentrations were determined using a two-site enzyme-linked immunosorbent assay and photometric measurement (Coaliza tPA and Coaset tPA, Chromogenix) [58]. Each sample was measured in duplicate,

and the mean value used for data analysis. Samples were encoded with a unique identifier so that the person performing the measurements was blinded to subject, time-point, drug and forearm from which the sample had been obtained.

5.7.6 Plasma viscosity

Plasma viscosity was determined at 37°C using a Beckman-Coulter Viscometer II (Beckman Coulter U.K. Limited, High Wycombe, UK), 0.5 ml sample volume, 0.38 × 200 mm capillary and 2.5 PSI (17.24 kPa) measuring pressure. Reproducibility with this method was good across a range of values; ± 0.03 mPa.s between 1.00 - 2.00 mPa.s, and ± 0.05 mPa.s between 2.01 - 5.00 mPa.s.

Chapter 6.

Development of Vehicle for Uric Acid Administration

6.1 Uric acid dissolution

6.1.1 Introduction

Uric acid is sparingly soluble in conventional aqueous media, for example 0.9% saline or 5% dextrose solutions. Therefore, a suitable vehicle was required so as to allow administration of sufficient quantities of uric acid to cause a significant increase in serum uric acid concentration after local and systemic administration. Uric acid has been found to be highly soluble in strong alkaline solutions, for example 0.1% sodium hydroxide, but these are too toxic to consider for human administration. Uric acid solubility depends heavily on pH. For example, saturating concentrations are 350-890 μmol/l at pH 5.0, but 9400-12000 μmol/l at pH 7.0 [92]. Other factors may improve uric acid solubility *in vivo*, including low sodium concentrations, the presence of urea, proteins and mucopolysaccharides.

Historical references describe high solubility of uric acid in lithium carbonate-based media and several authorities, including James Parkinson advocated their use as elixirs to prevent and treat gouty arthritis [277]. Uric acid has been given by systemic administration to dogs to explore its pharmacokinetics [278], and two previous reports describe systemic administration of uric acid to small numbers of patients with hypouricaemia, using an aqueous lithium carbonate vehicle [279, 280].

6.1.2 Aims

To explore the dissolution characteristics of uric acid in lithium carbonate and sodium hydrogen carbonate solutions.

6.1.3 Materials and methods

Lithium carbonate (FW 73.89, approximately 99%) and uric acid (FW 168.1, minimum 99%) were supplied as Ultrapure® preparations from Sigma-Aldrich Co. Ltd., Gillingham, Dorset, UK. Sodium hydrogen carbonate (FW 84.01, minimum 99.5%) was supplied from BDH Laboratory Supplies, Poole, Dorset, UK. Studies were performed using a flat-based 100 ml Pyrex conical flask and 2 cm magnetic stirrer (Fisher Scientific Ltd., Loughborough, Leicestershire, UK). Incremental

amounts of carbonate salt were added to 50 ml 4% dextrose (10 ml double distilled water and 40 ml sterile 5% dextrose solution) until saturation.

Uric acid was added to a lithium carbonate and sodium hydrogen carbonate solutions, in progressively decreasing increments until saturation. The pH of the solutions was measured using a standard pH-meter, calibrated using pH8 buffer, and recorded at intervals throughout the study.

6.1.4 Results

Table 9. pH of aqueous lithium carbonate

Li_2CO_3 (g)	Distilled water (ml)	5% dextrose (ml)	Li_2CO_3 (g/l)	Li_2CO_3 (mmol/l)	pH
0.02	10	40	0.4	5.42	10.1
0.04	10	40	0.8	10.83	10.3
0.06	10	40	1.2	16.25	10.4
0.08	10	40	1.6	21.66	10.4
0.10	10	40	2.0	27.08	10.5

Table 10. pH of aqueous sodium hydrogen carbonate

$NaHCO_3$ (g)	Distilled water (ml)	5% dextrose (ml)	$NaHCO_3$ (g/l)	$NaHCO_3$ (mmol/l)	pH
0.03	10	40	0.6	7.1	7.8
0.15	10	40	3.0	35.7	8.3
0.3	10	40	6.0	71.4	8.4
0.6	10	40	12.0	142.8	8.7
0.63	10	40	12.6	150.0	8.7

Table 11. Uric acid solubility in aqueous lithium carbonate

Li_2CO_3 (g)	80: 10 dextrose: water (ml)	Li_2CO_3 (g/l)	Saturation point: uric acid (mg)	Uric acid (mg/ml)	Uric acid (mmol/l)	pH
0.02	50	0.4	125.0	2.5	14.9	9.4
0.04	50	0.8	225.0	4.0	23.8	9.3
0.06	50	1.2	250.0	5.0	29.7	9.0
0.08	50	1.6	275.0	5.5	32.7	8.9
0.10	50	2.0	250.0	5.0	29.7	8.9

Table 12. Uric acid solubility in aqueous sodium hydrogen carbonate

$NaHCO_3$ (g)	80: 10 dextrose: water (ml)	$NaHCO_3$ (g/l)	Saturation point: uric acid (mg)	Uric acid (mg/ml)	Uric acid (mmol/l)	pH
0.03	50	0.6	<12.5	-	-	7.8
0.15	50	3.0	12.5	0.25	1.5	8.3
0.3	50	6.0	100.0	2.0	11.9	8.4
0.6	50	12.0	150.0	3.0	17.8	8.7
0.63	50	12.6	180.0	3.6	21.4	8.3

6.1.5 Discussion

Uric acid solubility was carbonate concentration and pH dependent. Higher concentrations of could be dissolved in lithium carbonate solution, to a maximum concentration of 5 mg/ml. As a safety precaution, to avoid precipitation, 4mg/ml was selected as the maximum concentration to be prepared for human administration in 4% dextrose/0.1% lithium carbonate solution.

6.2 Effect of uric acid solution on blood pH

6.2.1 Introduction

4% dextrose/0.1% lithium carbonate vehicle was found to have pH 10.4, partially buffered by the addition of uric acid (Section 6.1). The possibility of an effect on local pH *in vivo* might render the vehicle solution hazardous and non-viable for human administration due, for example, to peripheral vein irritation. Furthermore, changes in local serum pH *in vivo* might result in difficulty interpreting any haemodynamic responses to uric acid.

6.2.2 Aims

To examine the effects of 4% dextrose/0.1% lithium carbonate vehicle on *ex vivo* serum pH, in concentrations up to and exceeding those likely to be achieved *in vivo*.

6.2.3 Methods

A healthy male subject rested supine for 10 min, and 20 ml venous blood sample was drawn from a large antecubital fossa vein using an 18 G needle and aseptic technique. 5 ml whole blood was immediately added to each of 4 tubes containing potassium EDTA solution and 0, 0.1, 1.0 and 2.0 ml of 0.8% lithium carbonate solution, and gently mixed. After centrifugation at 1000 *g* for 20 minutes at 4°C, serum was separated and pH measured in triplicate.

6.2.4 Results

Table 13. Effect of 4% dextrose/0.1% lithium carbonate vehicle on plasma pH.

Vehicle (ml)	Blood (ml)	Relative concentration	pH 1	pH 1	pH 1	Mean pH	S.D.
0	5	-	7.5	7.5	7.6	7.53	0.06
0.1	5	1 in 50	7.5	7.6	7.6	7.57	0.05
1	5	1 in 5	7.6	7.7	7.7	7.67	0.06
2	5	1 in 2.5	7.7	7.6	7.63		0.06

6.2.5 Discussion

There is no clinically significant change in plasma pH following addition of 0.1ml, 1.0ml or 2.0ml of vehicle solution to 5ml of blood. In our forearm blood flow studies vehicle will be infused at a maximum of 1 ml/min to the brachial artery, and forearm blood flow is estimated at 50 ml/min. The volumes of vehicle solution were chosen to represent the effect of dilution where forearm blood flow is 50, 5 and 2.5 ml/min, so as to overestimate potential effects on pH.

The present findings indicated that, even if forearm blood flow is substantially less than anticipated, administration of vehicle solution will not significantly alter local plasma pH. Potential effects are likely to be minimised further *in vivo* due to the extensive buffering capacity of human blood and tissues.

Nonetheless, these studies do not fully exclude the possibility that intra-brachial or intravenous administration of vehicle, or uric acid in vehicle, might exert some effect on local or circulating acid-base balance. Therefore, the designs of subsequent clinical studies incorporated safety laboratory measurements that included serum bicarbonate concentrations, to determine whether administrations might have any effect on serum pH.

Furthermore, it was not possible to exclude the possibility that lithium might exert a confounding effect on vascular function *in vivo*. Therefore, serum lithium concentrations were closely examined in earlier studies involving systemic uric acid administration and, in later studies, a saline control arm was used in addition to vehicle alone to examine for possible confounding effects.

Chapter 7.

Intra-brachial Administration of Uric Acid in Healthy Subjects

7.1 Introduction

Uric acid could be dissolved in 4% dextrose/0.1% lithium carbonate vehicle solution (see Chapter 6). The following study was designed so that the potential effects of high local uric acid concentrations on vascular tone and blood flow could be examined in the forearm vascular bed, by means of direct intra-brachial administration of uric acid. Resting vascular tone is determined only in part by native endothelial function and is comparatively insensitive to the potential effects of high uric acid concentrations on endothelial function. Therefore, endothelial function was assessed directly by intra-brachial administration of acetylcholine, sodium nitroprusside and L-NMMA during co-administration of uric acid, or vehicle as control. The purpose of this study was to examine whether high uric acid concentrations might impair endothelial function in the forearm vascular bed, as has previously been shown with acute exposure to a number of established major cardiovascular risk factors [281, 282].

7.2 Methods

Six healthy men were recruited to a two-way randomised placebo controlled study. Subjects underwent insertion of a brachial artery needle, followed by intra-arterial saline administration for 20 min to allow baseline blood flow to be established. This was followed by infusion of 0, 0.5, 1.0, 2.0, 4.0 mg/min uric acid in 4% dextrose/0.1% lithium carbonate vehicle for 6 min at each dose, and 12 min at the maximum dose. Forearm blood flow was assessed at baseline, and during infusion of each concentration using venous occlusion plethysmography. Heart rate and blood pressure were recorded at baseline and during each infusion.

The rate of uric acid administration was selected on the basis of maximum solubility of 4 mg/ml in vehicle solution, and estimated resting forearm blood flow of 50 ml/min. The *a priori* study assumption was that delivery of uric acid 0.5, 1.0, 2.0 and 4.0 mg/min would cause an increase in local uric acid concentrations of 58, 117, 235 and 467 μmol/l respectively.

In a separate study, 10 healthy men were recruited to a two-way randomised placebo controlled study. Subjects underwent intra-arterial administration of saline for 30 min, to establish baseline blood flow, followed by ACh 7.5, 15 and 30 mmol/min, SNP 2, 4 and 8 mmol/min, and L-NMMA 2 and 4 μmol/min, where the order of infusion of ACh and SNP was randomised between subjects. Drug infusions were separated by 20 min saline to allow restoration of basal blood flow, and infused for 6 min at each dose. Uric acid 2.0 mg/min in 4% dextrose/0.1% lithium carbonate vehicle, or vehicle alone, were co-infused locally. 5 ml effluent venous blood was collected from each forearm during infusion, for uric acid measurement.

7.3 Results

Table 14. Baseline characteristics of study population. NM = not measured

Characteristic	**Mean ± SEM**	**Mean ± SEM**
Number/ male	6/6	10/ 10
Age (y)	29 ± 4	23 ± 1
Systolic BP (mmHg)	118 ± 8	110 ± 4
Diastolic BP (mmHg)	72 ± 7	68 ± 5
Heart rate (bpm)	62 ± 4	59 ± 2
Creatinine (μmol/l)	NM	84 ± 4
Glucose (mmol/l)	NM	4.6 ± 0.2
Cholesterol (mmol/l)	NM	4.1 ± 0.2
Body mass index (kg/m^2)	NM	23 ± 1
Uric acid (μmol/l)	NM	258 ± 13

7.3.1 Resting forearm blood flow

Neither vehicle nor uric acid administration had any effect on basal forearm blood flow (Figure 5). No effects on heart rate or systemic blood pressure were observed.

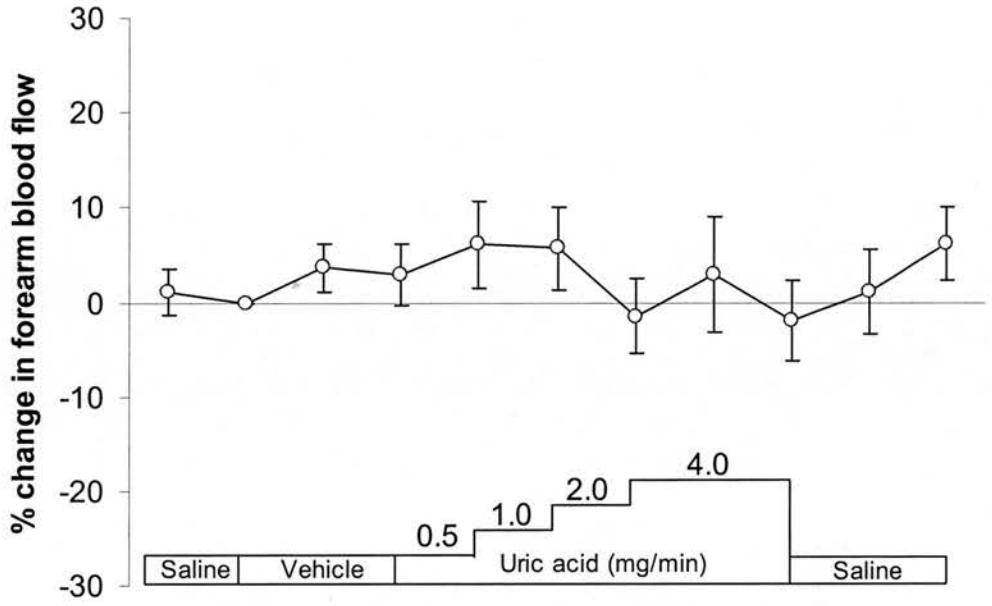


Figure 5. Mean ± SEM forearm blood flow, as infused: non-infused forearm ratio expressed as % change from baseline, during intra-brachial administration of uric acid 0-4 mg/min in vehicle (n = 6).

7.3.2 Venous effluent uric acid concentrations

Mean ± SEM venous effluent uric acid concentrations in the infused and non-infused forearms were 384 ± 7 and 280 ± 1 μmol/l during uric acid 2.0 mg/min administration ($p < 0.001$), and 290 ± 4 and 283 ± 1 μmol/l during vehicle administration respectively. This represented increases of 33 ± 3% and -1 ± 0% from baseline concentrations in the infusion arm, during uric acid 2.0 mg/min and vehicle administration respectively ($p < 0.001$).

7.3.3 Endothelial function

Dose-dependent changes in forearm blood flow were observed during administration of acetylcholine, sodium nitroprusside and L-NMMA (Figure 6). Uric acid administration did not have an effect on the responses to any vasoactive drugs ($p = 0.74$, 0.47 and 0.87 respectively).

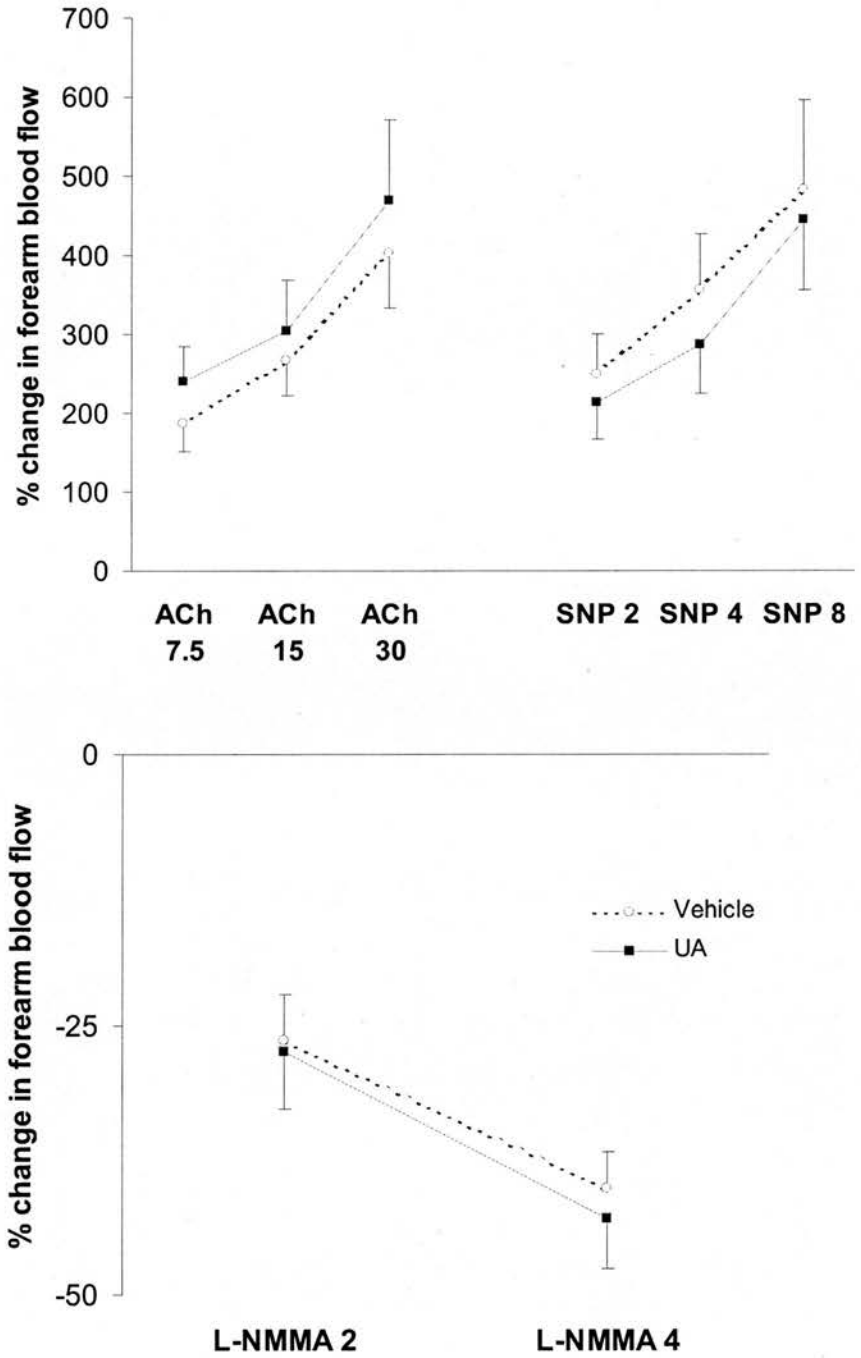


Figure 6: Forearm blood flow responses to acetylcholine 7.5-30 μg/min, sodium nitroprusside 2-8 μg/min and L-NMMA 2-4 μmol/min, as % change from baseline infused: non-infused forearm ratio, during local co-administration of 2 mg/min uric acid or vehicle (n = 10).

7.4 Discussion

Neither vehicle alone, nor uric acid in vehicle solution had any effect on basal forearm blood flow. This suggests that short-lived localised hyperuricemia does not have any direct effect on resting vascular tone in the forearm.

The dose-dependent responses to intra-brachial administration of vasoactive drugs was as expected in a healthy, young population who were free of any major cardiovascular risk factors. Lack of effect of uric acid co-administration on forearm blood flow responses to acetylcholine, sodium nitroprusside and L-NMMA suggests that high local uric acid concentrations do not influence endothelium-dependent and endothelium-independent nitric oxide-mediated responses, nor basal nitric oxide bioavailability. This suggests that high uric acid concentrations, at least in the acute setting, do not impair endothelial function in healthy subjects in whom oxidative stresses are low [69].

The rise in local uric acid concentrations associated with intra-brachial administration at 2.0 mg/min, although significant, was less than had been anticipated. This may reflect significantly higher basal forearm blood flow than had been allowed for using a prior estimate of 50 ml/min. It is possible that active uptake of uric acid by tissues might also account for lower venous concentrations than expected, but the short-term nature of the intra-brachial administration used in this study makes this a less plausible explanation. The modest increase evoked by local uric acid administration is a potential limitation of the present study. Effects on endothelial function might be more clearly observed if elevation to significantly higher concentrations had been attained. Localised administration of uric acid is unlikely to allow higher concentrations to achieved due to the limited solubility of uric acid, and the need to constrain intra-brachial infusion rates at no more than 1 ml/min.

No adverse effects were observed, confirming the feasibility of uric acid administration in a lithium carbonate based vehicle in a clinical research setting.

Chapter 8.

Intravenous Administration of Uric Acid in Healthy Subjects

8.1 Introduction

The studies described in Chapters 7 illustrate the lack of effect of intra-brachial administration of uric acid on basal blood flow and endothelial function in the forearm vascular bed. However, observations in the setting of localised uric acid administration are hampered by a number of important limitations. In particular, the technique allows only the effects of immediate or very short-term exposure to high uric acid concentrations to be evaluated. Furthermore, the potential rise in serum uric acid concentrations that can be achieved by this method is limited by constraints on intra-brachial infusion rate. In order to overcome these limitations, the present study was designed to examine the feasibility of raising systemic uric acid concentrations by means of intravenous infusion.

A secondary aim of the study was to evaluate the impact of uric acid administration on serum antioxidant capacity. Uric acid has potentially important antioxidant properties *in vitro*, and a number of observational studies have shown a positive correlation between uric acid concentrations and serum antioxidant capacity, suggesting that it may contribute to antioxidant defences *in vivo*. However, the potential for uric acid to behave as a pro-oxidant is also recognised (see Chapter 3.3.1). The effect of raising circulating uric acid concentrations on *in vivo* antioxidant defences is uncertain. Vitamin C was chosen as an active comparator because its effects have been more widely studied *in vivo* and *ex vivo*, and it too is an aqueous antioxidant of similar molecular weight to uric acid. The biological relevance of the antioxidant properties of vitamin C is better established. For example, vitamin C concentrations are lower in regular smokers and patients with diabetes or hypertension, and acute administration allows restoration of endothelial function in these groups.

In designing this study, particular consideration was given to a number of important safety issues related to the potential exposure to high uric acid and lithium concentrations.

8.2 Methods

8.2.1 Calculation of systemic uric acid dose

Intra-brachial administration of uric acid (total 237 mg) and vehicle caused mean ± SEM systemic uric acid concentrations to increase by 62 ± 13 and -4 ± 3 μmol/l respectively ($p < 0.001$), described in Chapter 7. Volume of distribution for administered uric acid was 22.6 ± 2.0 l, calculated using:

Volume of distribution = Administered dose ÷ concentration achieved

The same equation was used to determine that administration of uric acid 0.988 g would be required in order to achieve an increase of 260 μmol/l, representing an approximate doubling from baseline serum concentrations. For convenience, a systemic uric acid dose of 1000 mg uric acid was chosen. For the purpose of systemic administration, this was dissolved in 500 ml 4% dextrose/0.1% lithium carbonate as uric acid 2 mg/ml solution.

8.2.2 Safety considerations regarding lithium

Lithium is used in the treatment of bipolar affective disorder, and has a narrow therapeutic range. Therapeutic drug monitoring is implemented in clinical practice, so as to maintain serum lithium concentrations at 0.8-1.4 mmol/l. Toxic effects become more likely beyond this range, and particularly at concentrations greater than 2.0 mmol/l, including drowsiness, confusion, arrhythmia and, in severe cases, coma. Lithium is a simple cation that is widely distributed throughout bodily fluids and tissues and, therefore, serum concentrations were expected to decay rapidly due to extravascular distribution and renal elimination. Furthermore, the risks of lithium toxicity for any given serum concentration are substantially lower in individuals not previously exposed to lithium treatment because tissues are less likely to become saturated.

A calculation was performed to predict the maximum lithium concentration that might be attained after administration of 500 ml 4% dextrose/0.1% lithium carbonate vehicle as a bolus infusion. Lithium could be expected to distribute widely amongst the extravascular compartment. For safety reasons, the calculations considered the

possibility that lithium would be distributed within total body water compartment, extracellular fluid compartment and the intravascular space alone.

500 ml 0.1% lithium carbonate/4% dextrose
0.5 g Li_2CO_3 = 6.767 mmol (F.W. = 73.89 g)
Containing 13.534 mmol free lithium

Assumed Volume of Distribution	Compartment	Max. lithium (mmol/l)
40 l	Total body fluid	0.338
15 l	Extracellular	0.902
8 l	Intravascular	1.692

Based on distribution confined solely to the intravascular fluid compartment, the predicted maximum lithium concentration after bolus administration was marginally higher than the upper limit of the normal therapeutic range. Based on the more plausible situation that lithium would be more widely distributed, lower concentrations might be anticipated. However, as a safety precaution, administration of 500 ml 4% dextrose/0.1% lithium carbonate would be infused over 1 h rather than by bolus administration. The duration of exposure to high serum lithium concentrations was expected to be short, and vehicle administration was not expected to pose any significant clinical risk.

8.2.3 Study Protocol

Ten healthy subjects participated in this study. An exclusion criterion of serum uric acid concentration >300 mmol/l was applied, in addition to inclusion and exclusion criteria for healthy subjects outlined in Section 5.12. The study had a randomised placebo controlled double blind three-way crossover design, and study days were separated by at least 1 week. The study was conducted in a quiet, comfortable environment, maintained at 24 to 26°, within the Clinical Research Centre. Participants attended at 09:00 a.m. on each study day and were asked to avoid alcohol, caffeine and purine rich foods for twenty-four hours before.

An 18-G cannula was inserted into a large vein of each antecubital fossa using local anaesthesia and aseptic technique. The cannula in the non-dominant forearm allowed infusion over 1 h of 1000 mg uric acid (5952 μmol) in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, vehicle alone, or 1000 mg vitamin C (5682 μmol) in 500 ml 0.9% saline. 5 ml venous blood was drawn from the other cannula at baseline and 15 min intervals during infusion for measurement of uric acid and lithium concentrations. An additional 5 ml sample was collected for serum antioxidant capacity by chemiluminescence and 'Total Antioxidant Status' assays: samples were allowed to clot, centrifuged at 1000 *g* for 10 minutes at 4°C, decanted immediately and stored at -40°C before analyses. Additional blood samples at 15, 30, 60, 120, 300 and 1260 min after infusion allowed analysis of uric acid and lithium kinetics.

8.2.4 Kinetic analyses for uric acid and lithium

Standard linear and semi-logarithmic graphs representing serum lithium concentration versus time were plotted. Curve fitting by non-linear regression analyses was performed using Prism® software, version 3.0 for Windows (GraphPad Software Incorporated, San Diego, USA) [11]. F tests were used to compare best-fit curves generated by one and two exponent analyses, for each subject, and statistical significance was accepted at the 5% level.

8.3 Results

Table 15. Baseline characteristics of the study population at screening

Characteristic	Mean ± SEM
Number/ male	10/ 5
Age (y)	26 ± 1
Height (m)	1.69 ± 3
Weight (kg)	63 ± 3
Systolic blood pressure (mmHg)	106 ± 5
Diastolic blood pressure (mmHg)	69 ± 3
Serum urate (μmol/l)	227 ± 9
Serum cholesterol (mmol/l)	4.2 ± 0.2
Serum creatinine (μmol/l)	74 ± 4

Table 16. Safety laboratory data, presented as mean ± SD values

	Baseline	Post-infusion	+ 1 h	+ 21 h
Urea (mmol/l)	4.5 ± 1.5	4.3 ± 1.5	3.8 ± 1.6	3.9 ± 2.2
Creatinine (μmol/l)	76 ± 20	72 ± 19	67 ± 21	77 ± 26
Sodium (mmol/l)	140 ± 1	138 ± 1	140 ± 1	142 ± 1
Potassium (mmol/l)	3.8 ± 0.3	3.8 ± 0.3	3.8 ± 0.2	4.2 ± 0.2
Bicarbonate (mmol/l)	24 ± 2	24 ± 2	25 ± 2	25 ± 2

No adverse events were observed in any subject.

8.3.1 Uric acid pharmacokinetics

Mean ± SEM baseline serum uric acid concentration was 227 ± 9 μmol/l (95% reference range 120 to 420 μmol/l). Infusion of vehicle and uric acid caused serum concentrations to increase by -4 ± 2 and 307 ± 29 μmol/l respectively, to 220 ± 27 and 534 ± 29 μmol/l respectively at 1 h ($p < 0.001$).

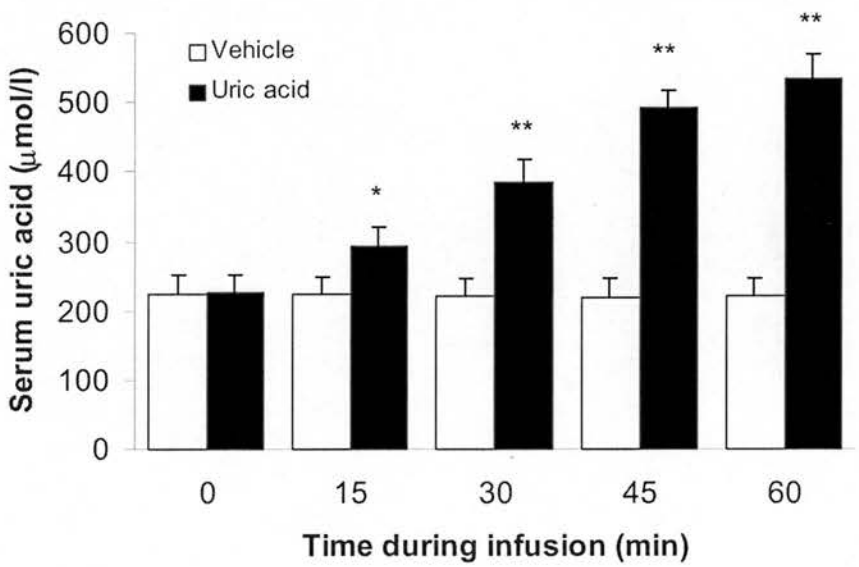


Figure 7. Mean ± SEM serum uric acid concentrations during intravenous administration of uric acid 1000 mg in vehicle or vehicle alone over 60 min. $p<0.001$ by ANOVA. $^{*}p < 0.05$, $^{**}p<0.005$ compared to vehicle by Students' two-tailed *t* test.

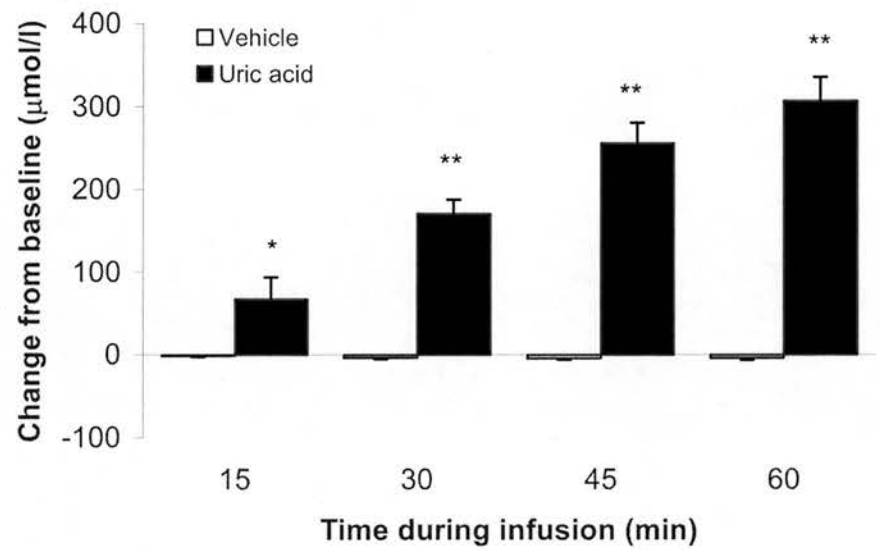


Figure 8. Mean ± SEM increment in serum uric acid concentration during systemic administration over 1 h of uric acid 1000 mg in vehicle or vehicle alone. $p<0.001$ by ANOVA. $^{*}p < 0.05$, $^{**}p<0.005$ compared to vehicle by Students' two-tailed *t* test.

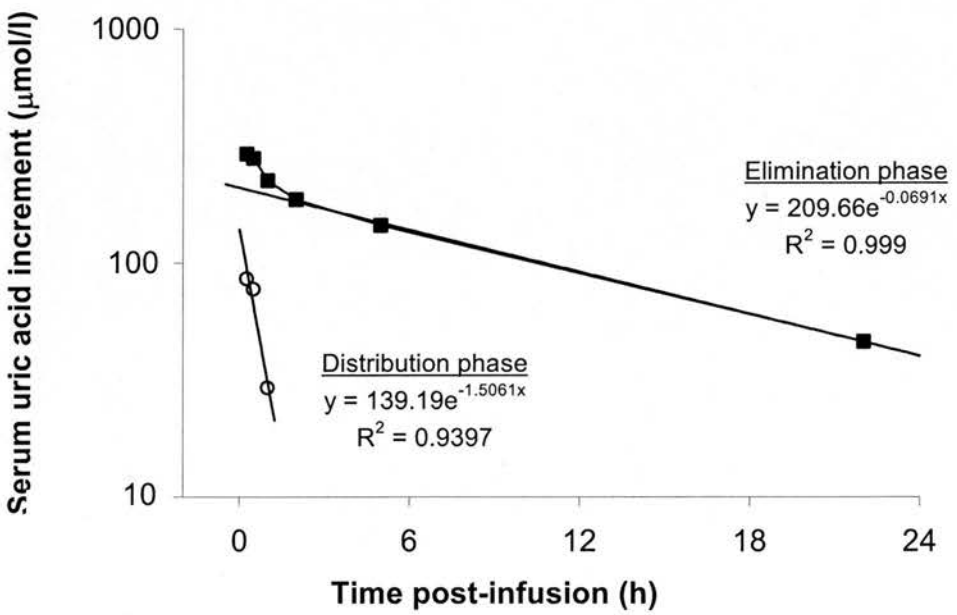


Figure 9. Increment in serum uric acid concentration exhibited a two-component decay, with mean elimination half-life of 10.8 h.

8.3.2 Antioxidant capacity

Administration of vehicle alone did not have any significant effect on serum antioxidant capacity, determined by either the chemiluminescence or 'Total Antioxidant Capacity' assay.

Administration of uric acid and vitamin C caused serum antioxidant capacity (by chemiluminescence) to increase by 139% and 20% from baseline respectively ($p < 0.001$ and $p = 0.56$ compared to vehicle). Administration of uric acid and vitamin C caused serum antioxidant capacity (by 'Total Antioxidant Status' assay) to increase by 23% and 7% from baseline respectively ($p < 0.001$ and $p = 0.65$ compared to vehicle).

The increase in serum antioxidant capacity caused by uric acid was significantly greater than that caused by vitamin C, as determined by chemiluminescence ($p < 0.001$) and 'Total Antioxidant Status' assay ($p < 0.001$).

Table 17. Mean ± SEM serum antioxidant capacity before and after administration of uric acid 1000 mg, vitamin C 1000 mg or vehicle, determined by two distinct antioxidant assays. $^{*}p < 0.05$, $^{**}p < 0.001$ compared to baseline; $^{‡}p < 0.05$, $^{‡‡}p < 0.001$ compared to vehicle.

		Baseline	**Post-infusion**	**Change**
TAS	Uric Acid	1235 ± 50	1520 ± 53**	284 ± 17‡‡
	Vitamin C	1288 ± 30	1379 ± 34*	92 ± 28‡
	Vehicle	1264 ± 47	1278 ± 43	14 ± 15
Chemiluminescence	Uric acid	384 ± 41	896 ± 69**	448 ± 84‡‡
	Vitamin C	410 ± 34	477 ± 33*	68 ± 34‡
	Vehicle	411 ± 35	401 ± 43	-9 ± 28

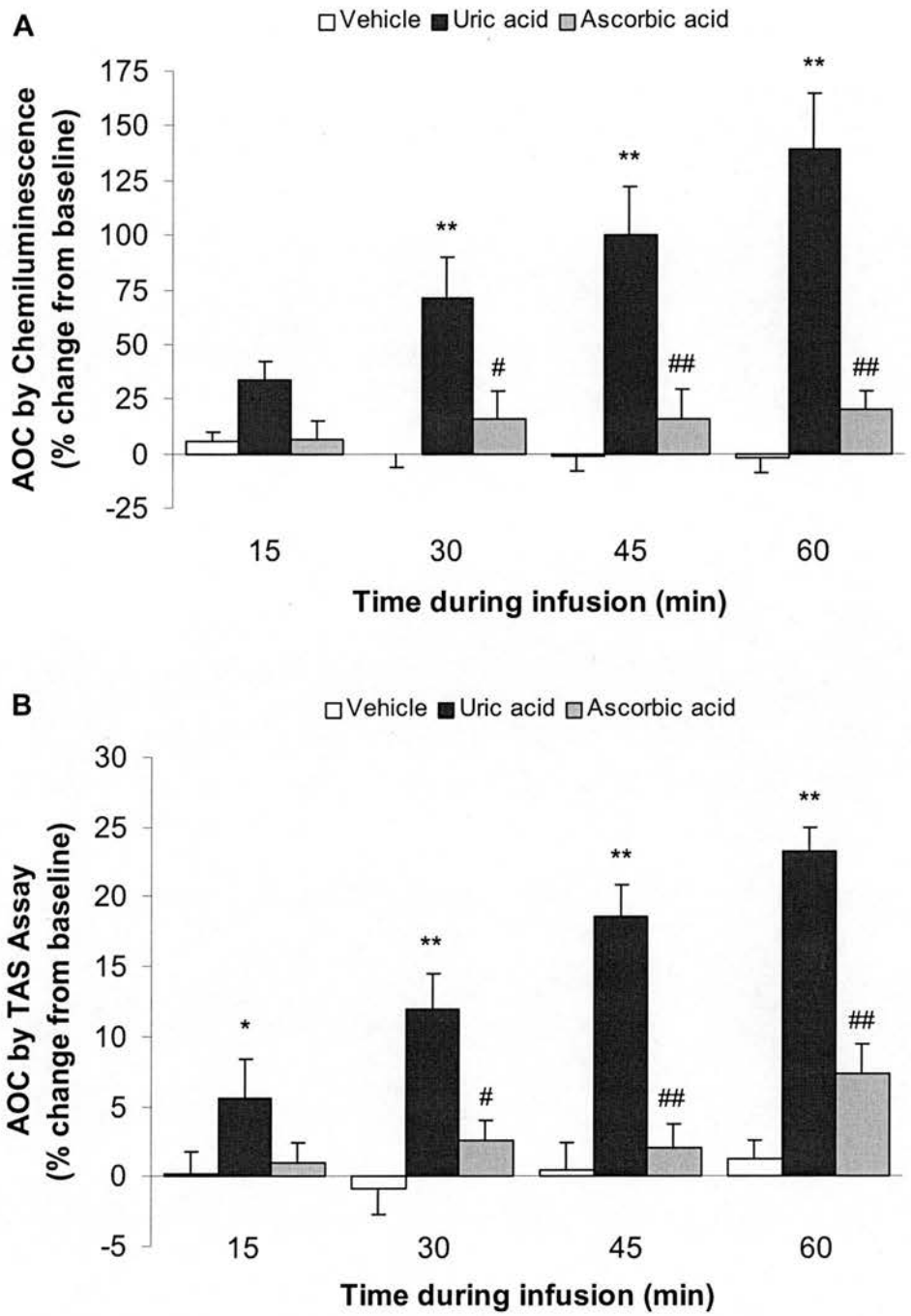


Figure 10. Mean ± SEM increment in serum antioxidant capacity during systemic administration over 1 h of uric acid 1000 mg, vitamin C 1000 mg or vehicle, determined by (A) chemiluminescence and (B) 'Total Antioxidant Status' assay. $^{*}p < 0.05$, $^{**}p<0.001$ between uric acid and vehicle, $^{\#}p < 0.005$, $^{\#\#}p < 0.001$ between uric acid and vitamin C by Students' two-tailed *t* test.

8.3.3 Lithium pharmacokinetics

Lithium carbonate infusion led to a time-dependent increase in serum lithium concentration, as shown in Figure 11. The mean ± SEM peak concentration immediately after administration was 0.93 ± 0.05 mmol/l. This was followed by a rapid decay that appeared consistent with a one or two-compartment model (Figure 12). The unweighted decay plot data were subjected to exponential and bi-exponential analyses, and compared to determine the best fit. The F value was > 1.0 in favour of a two-compartment model for data from each subject. The sum of squares, as a measure of distribution around the residuals, for the one and two compartment models were 1.313×10^{-3} and 7.37×10^{-6} respectively. F = 96.06 and p = 0.01 for the averaged data. Non-linear bi-exponential regression analysis of individual subject data found elimination half-life 7.8 ± 1.7 h, distribution phase half-life 37 ± 12 min, volume of distribution 43.4 ± 2.3 l, and clearance 5.3 ± 1.1 l/h.

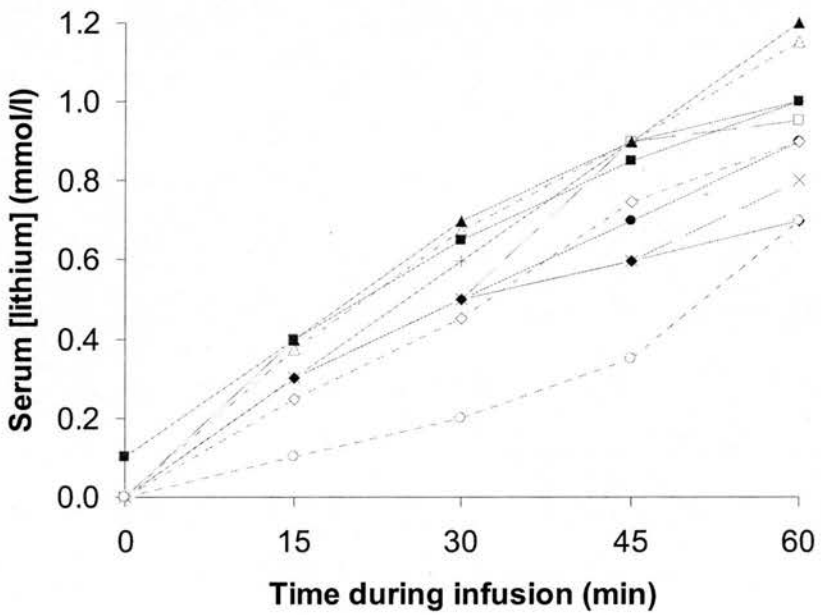


Figure 11. Time-dependent increase in serum lithium concentrations during administration of 500 ml 4% dextrose/0.1% lithium carbonate vehicle over 1 h in healthy subjects (n = 10).

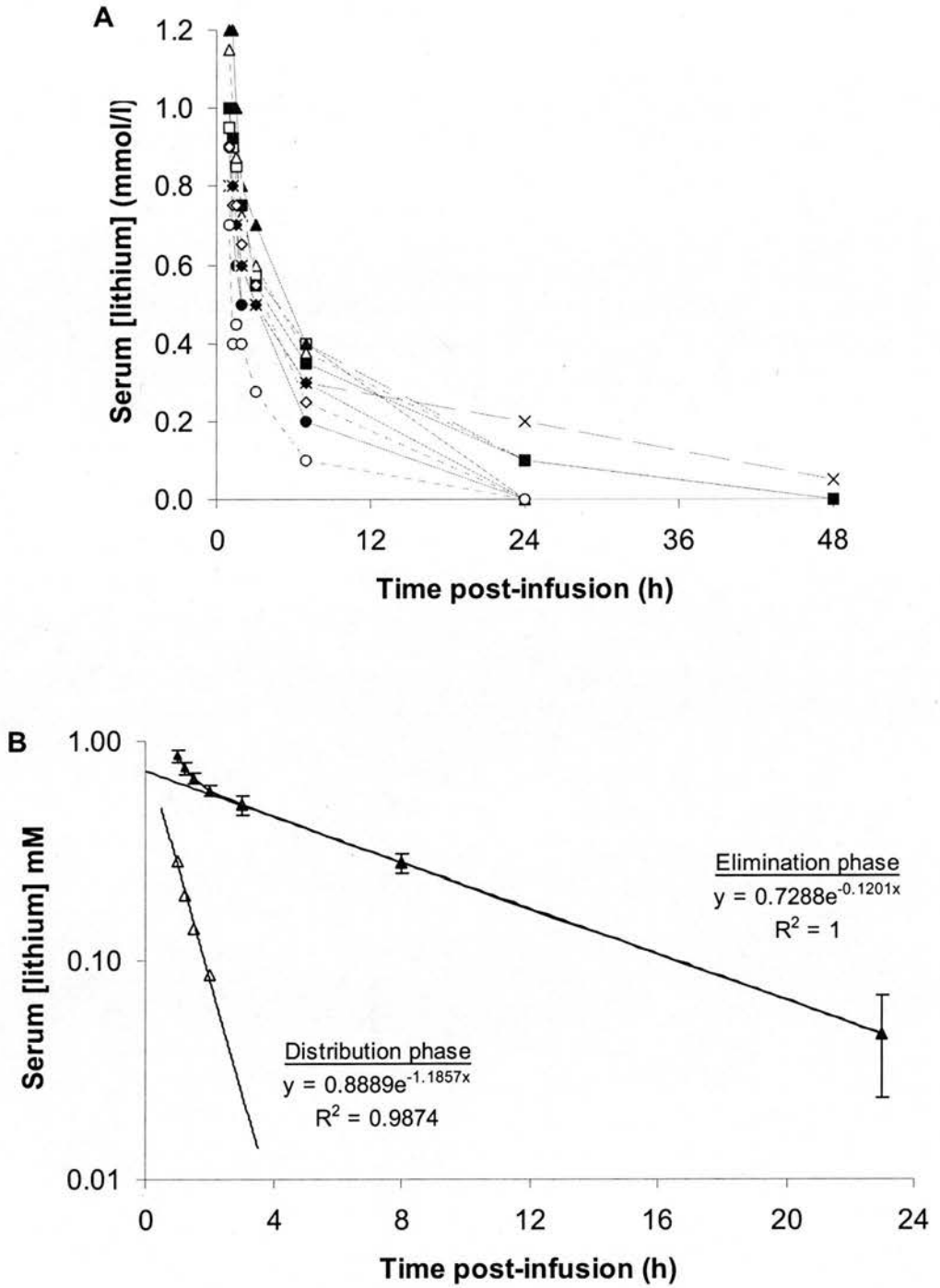


Figure 12. Decay of lithium concentrations after the end of administration of 500 ml 4% dextrose/0.1% lithium carbonate vehicle as (A) individual data on linear scale, and (B) mean data on logarithmic scale representing distribution and clearance components by bi-exponential analysis.

8.3.4 Systemic haemodynamic data

Systemic administration of uric acid was not associated with any significant haemodynamic effects. Administration of vehicle alone caused a modest reduction in cardiac output, which was not observed during administration of uric acid and vitamin C. There was a non-significant trend towards increased systemic vascular resistance and blood pressure from baseline across all three study groups.

Table 18. Mean ± SEM systemic haemodynamic variables before and immediately after intravenous administration of vehicle, vitamin C 1000 mg or uric acid 1000 mg.

		Vehicle	**Vitamin C**	**Uric Acid**
Heart rate	Before	59 ± 3	57 ± 3	54 ± 2
(bpm)	After	55 ± 2	58 ± 3	61 ± 4
Mean blood pressure	Before	78 ± 2	79 ± 2	80 ± 2
(mmHg)	After	81 ± 2	82 ± 2	84 ± 1
Cardiac output	Before	6.8 ± 0.5	6.8 ± 0.4	6.8 ± 0.3
(l/min)	After	6.1 ± 0.4*	6.5 ± 0.4	6.8 ± 0.4
Systemic vascular resistance	Before	11.9 ± 0.9	12.0 ± 0.8	12.1 ± 0.6
(l/min per mmHg)	After	13.7 ± 0.9	13.2 ± 0.8	12.7 ± 0.8

8.4 Discussion

No adverse clinical, biochemical or haemodynamic effects were observed following administration of uric acid 1000 mg. These observations suggest that high uric acid concentrations do not, at least in an acute setting, increase systemic vascular resistance or blood pressure.

Peak lithium concentrations obtained were within acceptable limits, and short-lived due to rapid distribution to extra-vascular compartments and renal clearance. No subject was exposed to lithium concentrations above the normal therapeutic range. These observations confirmed the feasibility and tolerability of systemic administration of uric acid in a lithium carbonate-based vehicle in a research setting.

This study found that raising serum uric acid concentrations, by means of direct administration, causes increased serum antioxidant capacity as determined by two methodologically distinct assays. The lithium carbonate-based vehicle solution had no direct effects on serum antioxidant capacity or uric acid concentration. These findings lend support to the view that high serum uric acid concentrations confer increased free radical scavenging capacity *in vivo*, and might be protective in the setting of oxidative stress. Acute administration of other naturally occurring antioxidants has previously been shown to confer a favourable effect on clinical outcome by preventing oxidative and free radical mediated tissue damage, for example in the setting of sepsis syndrome or after recent myocardial infarction [283, 284]. Based on the observed elimination half-life of administered uric acid, the conferred increase in antioxidant capacity is likely to persist for at least several hours after a single intravenous administration. Furthermore, the magnitude of the increased serum antioxidant capacity conferred by uric acid was substantially greater than that associated with vitamin C. This suggests that uric acid administration might be a more useful research tool for exploring the potential effects of aqueous antioxidants on vascular function.

Chapter 9.

Intravenous Administration of Uric Acid and Vascular Function in Healthy Subjects

9.1 Introduction

Having established the feasibility of raising circulating uric acid concentrations by means of direct systemic administration, the effects on endothelial function, large arterial stiffness and baroreceptor reflex sensitivity were examined. The premise underlying this study was that systemic administration of uric acid would allow vascular function to be examined in the presence of significantly higher serum concentrations than afforded by local uric acid administration. Furthermore, if high uric acid concentrations are capable of impairing endothelial function, then this might be more readily observed in the presence of more prolonged exposure after systemic administration than during transient elevations of local concentrations during intra-brachial uric acid administration.

Established major cardiovascular risk factors, such as regular smoking, diabetes mellitus, hypercholesterolaemia and hypertension, impair nitric oxide-dependent endothelial function. In vascular beds that contribute to systemic vascular resistance, this is characterised by impaired blood flow responses to endothelium-dependent vasodilators, while in large conduit arteries endothelial dysfunction manifests as increased large arterial stiffening and impaired baroreflex sensitivity.

9.2 Methods

Ten healthy subjects were recruited to a two-way randomised placebo controlled study of endothelial function. An 18-standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa, under local anaesthetic. Subjects underwent systemic administration of uric acid 1000 mg in 4% dextrose/0.1% lithium carbonate vehicle, or vehicle alone over 1 h via the cannula in the non-dominant forearm. 5 ml venous blood was drawn from the non-infused forearm cannula at baseline, at the end of the infusion, and 1 h after the end of the infusion for measurement of serum uric acid concentration. Immediately after completion of systemic infusion, endothelial function was studied using venous occlusion plethysmography and intra-brachial administration of acetylcholine, sodium nitroprusside and L-NMMA, as described in Chapter 5.

In a separate study, 8 healthy men were recruited to a three-way randomised placebo controlled study. An 18-standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa under local anaesthetic, and Portapres finger cuff and BoMed electrodes applied. Subjects rested supine for 30 min, then underwent systemic administration of 1000 mg uric acid in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, vehicle alone, or 0.9% saline over 1 h via the non-dominant forearm cannula. Electrocardiogram and Portapres signals were recorded for baroreflex sensitivity determination before and after systemic infusion, and blood pressure, cardiac index and pulse wave analysis measurements were recorded at baseline and 15 min intervals after commencement of the infusion. 5 ml venous blood was drawn via the non-infused forearm cannula at baseline and immediately after infusion for measurement of serum uric acid concentrations and safety laboratory investigations.

9.3 Results

No adverse events occurred in any subject.

Table 19. Mean ± SEM baseline characteristics of the study population

Characteristic	**Endothelial function study**	**Haemodynamic study**
Number/ male	10/ 6	8/ 8
Age (y)	24 ± 1	30 ± 4
Systolic BP (mmHg)	107 ± 6	108 ± 3
Diastolic BP (mmHg)	71 ± 4	72 ± 3
Heart rate (bpm)	58 ± 3	60 ± 2
Body mass index (kg/m^2)	22 ± 1	23 ± 0
Creatinine (μmol/l)	84 ± 6	86 ± 3
Glucose (mmol/l)	4.8 ± 0.1	4.7 ± 0.3
Cholesterol (mmol/l)	4.1 ± 0.2	4.8 ± 0.2
Uric acid (μmol/l)	252 ± 14	366 ± 12

9.3.1 Serum uric acid concentrations

Table 20. Mean ± SEM serum uric acid concentrations before, immediately after, and 1 hour after infusion of uric acid 1000 mg in 500 ml vehicle or vehicle alone. $^{*}p < 0.001$ compared to baseline. $^{‡}p < 0.001$ compared to vehicle administration.

Endothelial function study	**Uric Acid**		**Vehicle**	
	Values	Change from baseline	Values	Change from baseline
Baseline	227 ± 24	-	224 ± 27	-
Post-infusion	534 ± 37*	307 ± 29‡	220 ± 27	-3.9 ± 2.1
+ 1 h	452 ± 32*	225 ± 21‡	217 ± 27	-9.7 ± 10.5

Table 21. Mean ± SEM serum uric acid concentrations before and after infusion of uric acid 1000 mg, vehicle alone or saline. $^{*}p < 0.001$ compared to baseline. $^{‡}p < 0.001$ compared to saline administration.

Haemodynamics study	**Uric acid**	**Vehicle**	**Saline**
Baseline	370 ± 16	370 ± 17	336 ± 12
Post-infusion	627 ± 23*‡	361 ± 17	350 ± 19

9.3.2 Forearm blood flow responses

Intra-brachial administration of acetylcholine, sodium nitroprusside and L-NMMA caused dose-dependent changes in forearm blood flow, as shown in Figure 13. Prior systemic administration of uric acid did not significantly alter responses to the locally administered vasoactive drugs ($p = 0.87$, 0.65, and 0.38 respectively by ANOVA).

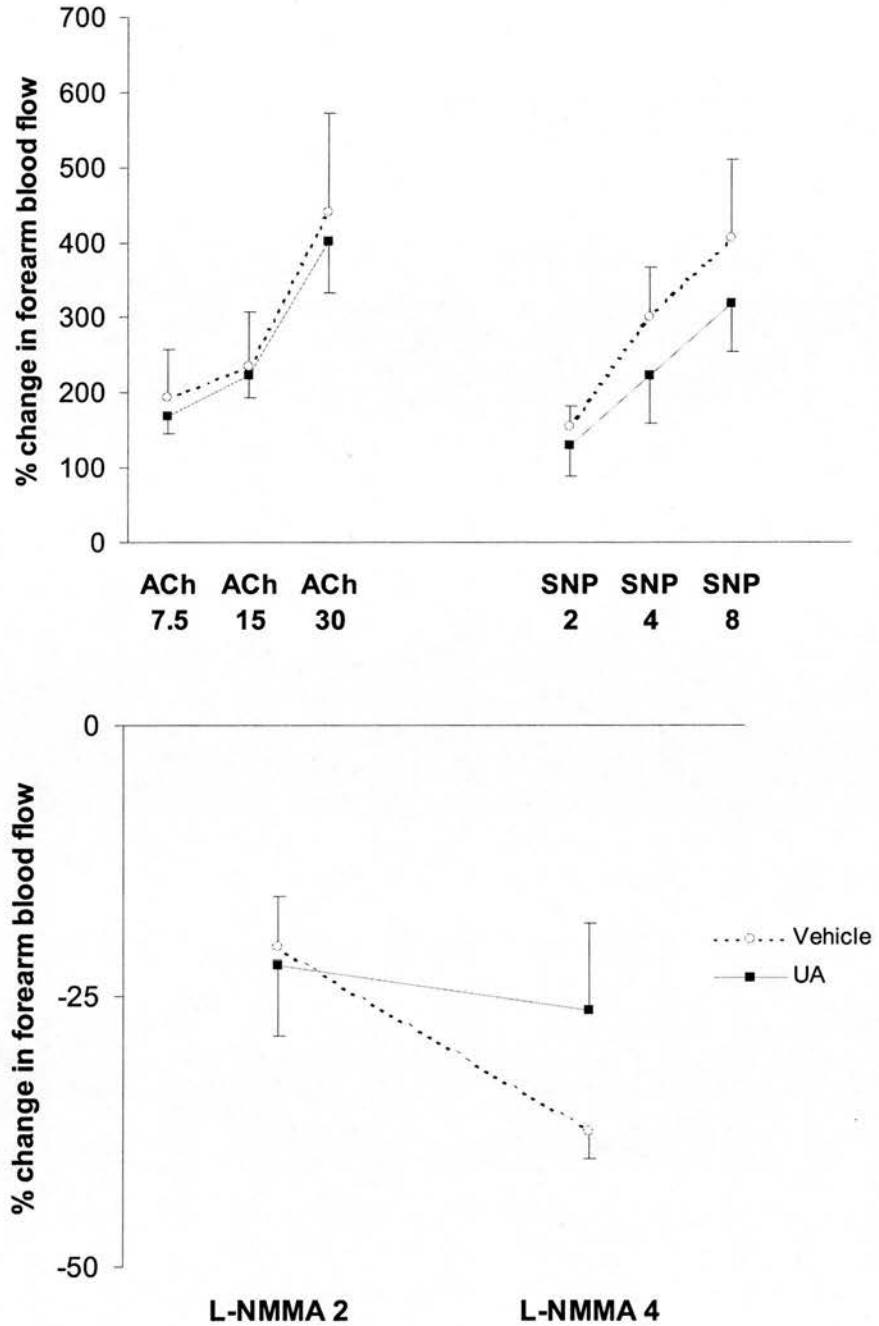


Figure 13. Forearm blood flow responses to acetylcholine 7.5-30 μg/min, sodium nitroprusside 2-8 μg/min and L-NMMA 2-4 μmol/min, as % change from baseline infused: non-infused forearm ratio, after systemic administration of uric acid 1000 mg or vehicle alone (n = 10).

9.3.3 Haemodynamic variables

Baseline values for augmentation index, haemodynamic variables and baroreceptor reflex sensitivity were similar between study arms. A non-significant trend was observed towards increased systemic vascular resistance and blood pressure in all three study arms. Uric acid elevation had no effect on any haemodynamic variables.

Table 22. Mean ± SEM central blood pressure, heart rate, augmentation index, cardiac index, systemic vascular resistance index and baroreflex sensitivity, before and after intravenous 0.9% saline, vehicle and uric acid 1000 mg over 1 h, and mean and 95% confidence intervals for change from baseline (Δ) at 1h.

	Saline		**Vehicle**		**Uric acid**	
	Before	After	Before	After	Before	After
SBP (mmHg)	96 ± 3	97 ± 3	93 ± 2	97 ± 5	96 ± 4	100 ± 4
DBP (mmHg)	61 ± 2	65 ± 2	63 ± 3	68 ± 3	62 ± 4	71 ± 3
HR (bpm)	55 ± 3	53 ± 3	57 ± 4	58 ± 3	58 ± 3	59 ± 2
AIx (%)	-6.7 ± 6.0	-3.1 ± 6.1	-2.6 ± 6.0	-1.1 ± 6.1	-3.6 ± 5.1	-6.1 ± 6.2
CI (l/min/m^2)	3.3 ± 0.2	3.0 ± 0.2	3.3 ± 0.4	3.3 ± 0.3	3.1 ± 0.2	3.2 ± 0.3
SVRI (au)	12.0 ± 1.0	13.7 ± 0.9	10.2 ± 1.5	11.7 ± 2.0	12.3 ± 1.0	13.0 ± 1.0
BRS_{SEQ}	22.3 ± 4.7	21.2 ± 4.0	19.7 ± 4.9	22.8 ± 5.0	21.8 ± 4.4	20.3 ± 4.4
BRS_{SPEC}	24.3 ± 5.8	19.4 ± 3.4	22.2 ± 6.6	26.8 ± 6.4	23.4 ± 5.0	21.7 ± 5.9

	Saline		**Vehicle**		**Uric acid**	
	Mean Δ	Δ 95% CI	Mean Δ	Δ 95% CI	Mean Δ	Δ 95% CI
SBP (mmHg)	1	-6 - 8	3	-3 - 9	3	-6 - 12
DBP (mmHg)	3	-2 - 8	1	-4 - 6	6	-2 - 14
HR (bpm)	-1	-5 - 7	-1	-6 - 4	0	-5 - 5
AIx (%)	3.5	-0.6 - 7.6	1.1	-2.7 - 4.9	2.4	-1.5 - 6.3
CI (l/min/m^2)	-0.1	-0.5 - 0.3	0.2	-0.2 - 0.6	0.0	-0.3 - 0.3
SVRI (au)	0.9	-0.3 - 2.1	1.2	-0.3 - 2.7	0.3	-0.8 - 1.4
BRS_{SEQ}	0.5	-1.3 - 2.3	0.8	-0.6 - 2.2	-0.5	-1.7 - 0.7
BRS_{SPEC}	-1.6	-3.7 - 0.5	2.2	-0.2 - 4.6	-1.4	-7.1 - 4.3

9.3.4 Safety laboratory variables

Table 23. Biochemical variables measured immediately before and after systemic administration of 0.9% saline, vehicle and uric acid 1000 mg, as mean ± SEM.

	Saline		Vehicle		Uric acid	
	Before	After	Before	After	Before	After
Sodium (mmol/l)	142 ± 0	141 ± 0	143 ± 1	140 ± 0	143 ± 0	140 ± 0
Potassium (mmol/l)	4.0 ± 0.1	4.3 ± 0.1	4.0 ± 0.1	4.2 ± 0.2	4.0 ± 0.1	4.1 ± 0.1
Urea (mmol/l)	5.3 ± 0.5	4.9 ± 0.6	4.8 ± 0.4	4.5 ± 0.4	5.2 ± 0.6	4.9 ± 0.5
Creatinine (μmol/l)	86 ± 4	84 ± 4	88 ± 3	87 ± 3	85 ± 4	85 ± 4
Cholesterol (mmol/l)	4.5 ± 0.4	4.1 ± 0.4	4.2 ± 0.4	3.9 ± 0.4	4.6 ± 0.4	4.2 ± 0.4
Triglycerides (mmol/l)	1.0 ± 0.2	0.9 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	1.1 ± 0.1	1.0 ± 0.1
Lithium (mmol/l)	-	0.0 ± 0.0	-	0.6 ± 0.1	-	0.6 ± 0.1

9.4 Discussion

As anticipated, intravenous administration of uric acid caused a substantial increase in circulating concentrations, which was of a similar magnitude to that observed in earlier studies, and persisted throughout the period when endothelial function was examined. Despite the substantial increase in circulating uric acid concentrations, local forearm blood flow responses to intra-brachial acetylcholine, sodium nitroprusside and L-NMMA were not altered. These findings indicate that high systemic uric acid concentrations do not influence constitutive nitric oxide bioavailability or stimulated endothelial function in the forearm vascular bed, consistent with the observations in the setting of local hyperuricaemia.

These findings are unlike the effects of acute exposure to high levels of other major cardiovascular risk factors, for example glucose, lipids, blood pressure and smoking, which have all been found to impair endothelial function, at least temporarily [281, 282]. This suggests that high uric acid concentrations do not mediate cardiovascular risk through impaired endothelial function. If indeed they are causally related to cardiovascular risk, then it appears likely that mechanisms other than impairment of endothelial function may be important.

Baseline haemodynamic measurements were consistent with values previously reported in healthy young subjects free of major cardiovascular risk factors. As anticipated, intravenous administration of uric acid caused a significant increase in circulating uric acid concentrations. High serum uric acid concentrations had no effect on augmentation index or baroreflex sensitivity, the latter determined by two methodologically discreet tests. These findings indicate that exposure to high circulating concentrations of uric acid does not impair normal large arterial compliance, at least in the acute setting. Similarly, no effect of hyperuricaemia was observed on central systolic or diastolic blood pressure. These observations are distinct from those reported in the presence of established major cardiovascular risk factors. For example, patients with diabetes mellitus and hypercholesterolaemia have higher augmentation index values than healthy people [42, 43]. This finding is indicative of increased large arterial stiffening, which might contribute to cardiovascular risk due to the consequent increase in systolic and lowering of diastolic central blood pressures [48].

Hyperuricemia co-exists with impaired large artery compliance in several disease states characterized by reduced vascular nitric oxide bioavailability, for example type 2 diabetes mellitus [31]. The present findings suggest that this may be a passive association, rather than caused by the presence of high serum uric acid concentrations. Alternatively, the apparent association between type 2 diabetes and large arterial stiffness might be accounted for by co-existent hypertension, and this needs further exploration in future studies.

Previous reports of hyperuricaemia in animal models had suggested a possible causal relationship between uric acid and elevated blood pressure, mediated through increased systemic vascular resistance [133]. The present findings did not support the possibility that a similar mechanism is operating in humans. The lack of effect of high serum uric acid concentrations on systemic vascular resistance and blood pressure is consistent with the observations reported in Chapter 8. A limitation is that the small number of subjects included, and the comparatively wide 95% confidence intervals do not fully exclude the possibility of a small haemodynamic effect of acute

hyperuricaemia. A further limitation of the present study design is that only the acute haemodynamic responses to hyperuricaemia could be observed. Therefore, the possibility that chronic exposure to high circulating uric acid concentrations might increase systemic blood pressure cannot be refuted.

Chapter 10.

Intravenous Administration of Uric Acid and Platelet Aggregation, Plasma Viscosity, and Tissue Plasminogen Activator

10.1 Introduction

Increased platelet aggregability has long been proposed as one plausible mechanism by which high uric acid concentrations might predispose to increased cardiovascular risk. A number of *in vitro* and observations in animal models have fuelled speculation that such a mechanism might operate within the human cardiovascular system, although a small number of exploratory clinical studies have yielded conflicting reports, as discussed in Section 3.3. A limitation associated with the direct addition of uric acid to whole blood *in vitro* is that hyperuricaemia might mediate effects on platelet aggregation through the release of other tissue factors. One of the major drawbacks to examining the effects of *in vivo* hyperuricaemia on platelet aggregability has been the lack of means to raise serum uric acid concentrations sufficiently to address the hypothesis. Therefore, having established the viability of uric acid administration as a means substantially raising serum concentrations, the present study sought to evaluate the potential effects on platelet aggregability.

Secondary study aims were to evaluate the effects of high serum uric acid concentration on other potential mechanisms related to thrombosis, through which uric acid might contribute to cardiovascular risk. Haemorheological measurements have received comparatively little attention, but are closely related to the development of atheroembolism, and are believed to contribute to cardiovascular risk in the setting of any one of a number of established cardiovascular risk factors. Additionally, endothelium-derived tissue plasminogen activator is believed to play an important role in regulating thrombosis *in vivo*, and impaired tissue plasminogen activator bioavailability appears to be an important mechanism underlying increased thrombosis risk in regular smokers (plasma viscosity and tissue plasminogen activator are discussed in greater depth in Chapter 1). A functional dichotomy is recognised between endothelium-dependent nitric oxide-mediated vasodilatation and endothelium-dependent tissue plasminogen activator release. For example, acetylcholine stimulates endothelial release of nitric oxide, but not tissue plasminogen activator, whereas bradykinin is a potent stimulus for endothelial tissue plasminogen activator release and less so nitric oxide. Therefore, the apparent lack of

effect of hyperuricaemia on endothelium-dependent nitric oxide bioavailability (as discussed in earlier Chapters) does not exclude the possibility that high serum uric acid concentrations might influence other aspects of endothelial function such as the release of tissue plasminogen activator in response to bradykinin.

The potential effects of high serum uric acid concentrations on plasma viscosity and tissue plasminogen activator have not been examined in previous studies.

10.2 Protocols

10.2.1 Plasma viscosity and platelet aggregation

Ten healthy men were recruited to a randomised double blind three-way crossover study. An 18-gauge standard venous cannula was inserted into a large vein in each antecubital fossa using local anaesthetic (1% lidocaine). Uric acid 1000 mg in 500 ml vehicle, 500 ml vehicle alone, or 500 ml 0.9% saline was infused via the non-dominant forearm cannula over 1 h. Venous blood was drawn via the non-infused forearm cannula immediately before and after infusion: 5 ml was collected in a serum gel tube (Sarstedt Ltd., Leicester, UK) for measurement of serum uric acid concentration; 10 ml was collected into a Sarstedt tube containing potassium EDTA for measurement of plasma viscosity; 30 ml was collected into evacuated tubes containing trisodium citrate (0.8 ml/10 ml) for measurement of platelet aggregation.

Samples for platelet aggregation were processed and analysed immediately after collection. Each was centrifuged at 120 *g* for 10 min, and platelet-rich plasma was aspirated. The remaining sample was centrifuged at 800 *g* for 10 min, and platelet poor plasma was aspirated. Platelet counts were obtained using a Beckman-Coulter Act-8 Counter (Beckman-Coulter U.K. Limited, High Wycombe, England). Platelet-rich plasma samples were diluted with platelet poor plasma to achieve a final concentration of 200×10^9/l. Aggregation studies were performed using a standard optical technique (Chronolog Ca560 aggregometer; Labmedics, Stockport, England), and samples were pre-warmed to 37°C before analyses. The maximal aggregation responses to ADP 0, 2, 4, 6, 8 and 10 μM obtained within 7 min were recorded and expressed as a percentage of response to ADP 100 μM in each subject. Repeated-

measures analysis of variance was used to identify differences in response between uric acid, vehicle and saline.

10.2.2 Forearm blood flow responses and tissue plasminogen activator

Eight healthy men were recruited to a randomised double blind two-way crossover study. An 18-standard gauge cannula was inserted into a suitable vein in each antecubital fossa, and a 27-standard gauge needle inserted into the brachial artery of the non-dominant arm using local anaesthesia and aseptic technique. Uric acid 1000 mg in 500 ml vehicle or 500 ml vehicle alone saline were infused intravenously, over 1 h, via the venous cannula in the non-dominant forearm.

A 5 ml venous blood sample was collected from the non-infused forearm cannula before and after infusion for measurement of serum uric acid concentration.

Immediately after infusion, subjects underwent intra-brachial administration of acetylcholine 7.5, 15 and 30 μg/min, sodium nitroprusside 2, 4 and 8 μg/min, and bradykinin 30, 100 and 300 ng/min. Each drug dose was infused for 6 min, and the rate of infusion was kept constant at 1 ml/min throughout. The order of intra-brachial drug infusion was randomised between subjects, and saline was administered for at least 20 min between drugs to allow restoration of basal blood flow. Blood flow was measured in both forearms by venous occlusion plethysmography during the last 3 min of each dose. Immediately after infusion of each dose, a 6 ml venous blood sample was drawn from infused and non-infused forearm cannulae into separate collecting tubes containing acidified buffered sodium citrate 0.105 mmol/l (Stabilyte, Biopool, Umea, Sweden), centrifuged at 2000 *g* for 20 min at 4°C. Plasma was decanted, and stored at -70°C prior to analysis of tissue plasminogen activator concentration.

10.3 Results

Table 24. Baseline characteristics of the study populations as mean ± SEM.

Characteristic	Plasma viscosity & platelet aggregation	Forearm blood flow and TPA
Number/ male	10/ 6	8/ 3
Age (years)	35 ± 2	27 ± 1
Height (m)	1.74 ± 0.02	1.74 ± 0.01
Weight (kg)	74 ± 4	70 ± 2
Body mass index (kg/m^2)	24.4 ± 0.8	22.1 ± 0.6
Heart rate (min^{-1})	71 ± 3	61 ± 2
Systolic blood pressure (mmHg)	123 ± 3	111 ± 3
Diastolic blood pressure (mmHg)	75 ± 2	64 ± 2
Serum creatinine (μmol/l)	77 ± 5	66 ± 5
Serum cholesterol (mmol/l)	4.6 ± 0.3	4.2 ± 0.3
Plasma glucose (mmol/l)	6.8 ± 1.4	5.8 ± 1.2
Serum urate (μmol/l)	307 ± 24	234 ± 19
Haematocrit (l/l)	0.42 ± 0.01	-
Platelet count ($\times 10^9$/l)	239 ± 14	-

10.3.1 Serum uric acid concentrations

Table 25. Mean ± SEM serum uric acid concentrations before and after systemic administration of uric acid 1000 mg in vehicle, vehicle and 0.9% saline. $^*p < 0.001$ compared to baseline.

		Baseline	Post-infusion
Plasma viscosity & platelet aggregation study	Uric acid	307 ± 27	585 ± 28*
	Vehicle	308 ± 20	313 ± 19
	Saline	306 ± 24	295 ± 23
Forearm blood flow and tissue plasminogen activator study	Uric acid	233 ± 17	528 ± 26*
	Vehicle	237 ± 20	224 ± 15

10.3.2 Plasma viscosity

Table 26. Mean ± SEM plasma viscosity measurements (mPa.s) before and after administration of uric acid 1000 mg, vehicle and 0.9% saline.

	Baseline	Post-infusion
Saline	1.56 ± 0.06	1.54 ± 0.08
Vehicle	1.54 ± 0.06	1.55 ± 0.05
Uric acid	1.55 ± 0.07	1.54 ± 0.07

10.3.3 Platelet aggregation

Dose-dependent aggregation responses to ADP were observed in all subjects. The dose-response relationship was not altered by prior administration of uric acid or vehicle ($p = 0.78$ and $p = 0.59$ compared to saline administration).

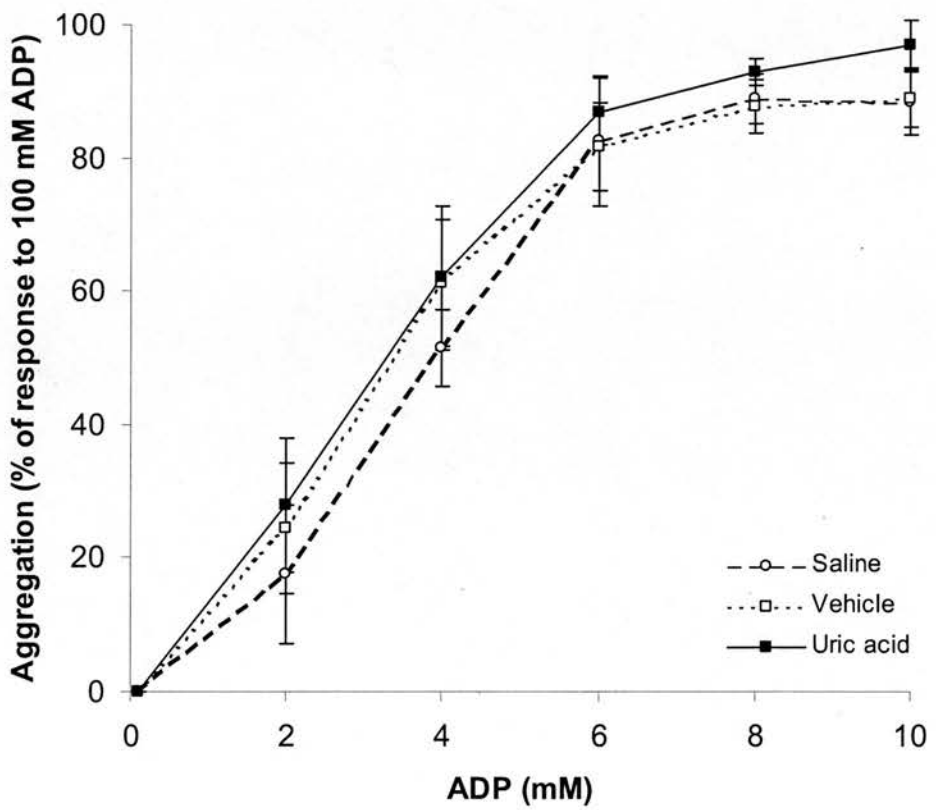


Figure 14. Mean ± SEM aggregation responses to ADP after administration of uric acid 1000 mg, vehicle and saline, as percentage of the response to 100 μM ADP.

10.3.4 Forearm blood flow responses

Intra-brachial administration of acetylcholine, sodium nitroprusside and bradykinin caused dose-dependent blood flow increases in the infused forearm. Administration of uric acid did not have a significant effect on the responses to any vasoactive drug ($p = 0.84$, $p = 0.68$ and $p = 0.91$ respectively).

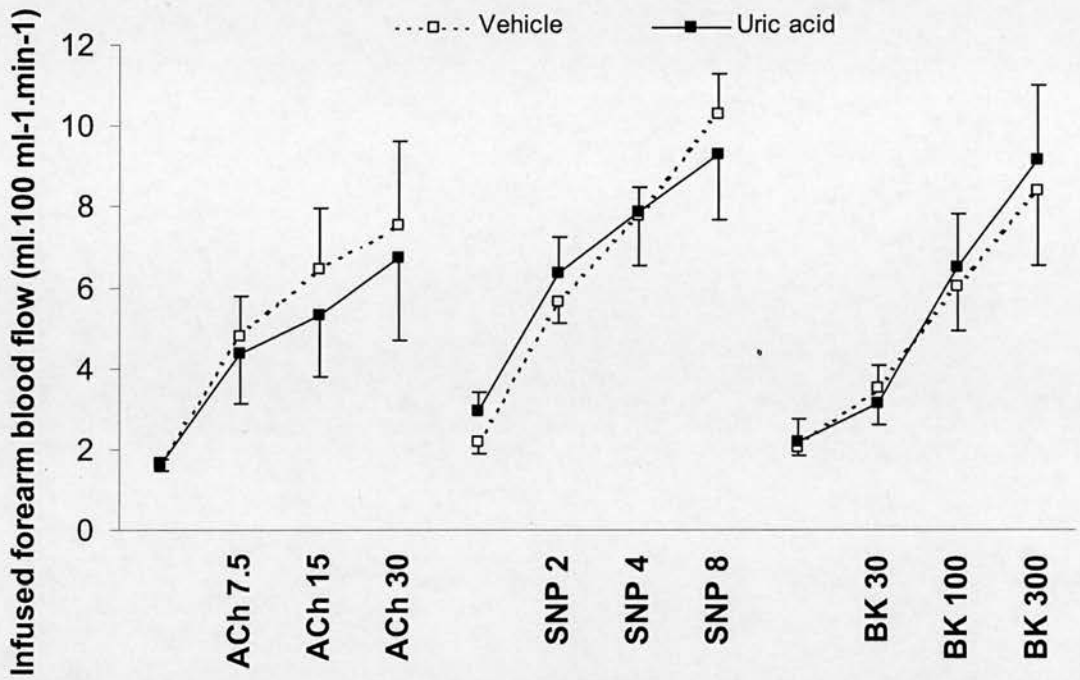


Figure 15. Forearm blood flow responses to intra-brachial acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and bradykinin (BK) 30-300 ng/min after systemic administration of uric acid 1000 mg or vehicle.

10.3.5 Tissue plasminogen activator

Intra-brachial administration of bradykinin caused a dose-dependent increase in net release of tissue plasminogen activator, whereas acetylcholine and sodium nitroprusside had no effect. Systemic administration of uric acid did not alter tissue plasminogen activator responses to bradykinin, acetylcholine or sodium nitroprusside, as shown overleaf.

Table 27. Mean ± SD net t-PA release during intra-brachial administration of acetylcholine 7.5-30 μg/min, sodium nitroprusside 2-8 μg/min and bradykinin 30-300 ng/min, after systemic administration of uric acid 1000 mg or vehicle. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.005$ compared to baseline.

	Net t-PA release ($ng.100ml^{-1}.min^{-1}$)	
	Vehicle	**Uric acid**
Baseline	0.51 ± 0.11	0.19 ± 0.23
ACh 7.5 μg/min	-0.06 ± 0.23	-0.06 ± 0.24
ACh 15 μg/min	0.43 ± 0.29	2.10 ± 1.28
ACh 30 μg/min	0.71 ± 0.33	1.61 ± 0.62
Baseline	-0.14 ± 0.40	0.36 ± 0.72
SNP 2 μg/min	-0.16 ± 0.27	-0.85 ± 0.81
SNP 4 μg/min	-0.18 ± 0.37	1.50 ± 0.81
SNP 8 μg/min	-0.55 ± 0.60	-0.43 ± 0.60
Baseline	0.78 ± 0.39	-0.13 ± 0.41
BK 30 ng/min	2.51 ± 0.52*	1.65 ± 0.43**
BK 100 ng/min	7.60 ± 2.33***	5.72 ± 1.62***
BK 300 ng/min	14.40 ± 2.83***	15.7 ± 3.23***

10.4 Discussion

Haematocrit and platelet count values were normal in the study population. High uric acid concentrations *in vivo* did not manifest any effects on platelet aggregation determined using an *ex vivo* method. This suggests that the observed relationship between hyperuricaemia and increased platelet aggregability is non-causal, and might arise through associations with other factors. The present findings contrast with previously reported effects of vitamin C, another aqueous antioxidant with similar molecular weight to uric acid. Acute oral administration of vitamin C has been shown to inhibit *ex vivo* platelet aggregation in healthy subjects [285]. Given that high serum uric acid concentrations confer powerful antioxidant properties within human serum, the previously observed effects of vitamin C appear unlikely to

be explained by its antioxidant properties alone, and other mechanisms may be important.

High uric acid concentrations had no discernable effect on plasma viscosity and endothelium-dependent release of tissue plasminogen activator. This suggests that uric acid is unlikely to predispose to thrombosis through either mechanism. Increased plasma viscosity has been established as an important risk factor in patients with hypertension, peripheral vascular disease and ischaemic heart disease [86-88]. Previous studies have shown an association between high serum uric acid concentrations and hypercoaguable states, and it remains possible that this relationship is reflective of other mechanistic links, or that the association is non-causal.

Chapter 11.

Intravenous Administration of Uric Acid and Oxidative Stress During Acute Aerobic Exercise

11.1 Introduction

Moderately intense physical exercise increases oxygen utilisation, and causes excess oxygen-derived free radical liberation through mitochondral lipid peroxidation, neutrophil degranulation, and up-regulation of xanthine oxidase activity, which liberates superoxide (O_2•). Intense physical exercise provides a model for studying the effects of acute oxidative stress *in vivo*, and has been shown to increase susceptibility of low-density lipoprotein to oxidation, and impair endothelial-dependent vasodilatation in the forearm vascular bed [286, 287]. Oxidative stress during acute exercise is characterised by elevated circulating 8-*iso*-$PGF_{2\alpha}$ concentrations, which has provided an opportunity to examine the effects of antioxidant administration on free-radical activity *in vivo* [288]. Supplementation with vitamin C or E increases resistance to exercise-induced lipid peroxidation in healthy individuals [289]. However, beta-carotene does not reduce oxidative stress during acute physical exercise [290], and combined administration of vitamin C and N-acetyl-cysteine paradoxically increase 8-*iso*-$PGF_{2\alpha}$ concentrations after acute eccentric exercise, despite increased serum antioxidant capacity [291]. Glutathione and ubiquinol supplementation reduce oxidative stress in animal models of acute exercise [292], but their effects have not been fully characterised in humans.

Uric acid becomes oxidised in skeletal muscle during high intensity exercise [293]. Intracellular uric acid concentrations are rapidly replenished by uptake from plasma after exercise [294]. A previous study found a significant inverse relationship between serum uric acid concentrations and oxidative stress during acute aerobic exercise [295]. These observations suggest that high uric acid concentrations could confer protection against free radical activity *in vivo*, and indicate that uric acid may be of biological importance in the setting of acute oxidative stress. The present study aimed to characterise the effects of elevated serum uric acid concentration on oxidative stress induced by acute physical exercise, reflected by plasma 8-*iso*-$PGF_{2\alpha}$ concentrations.

11.2 Methods

Healthy subjects were enrolled in a randomised two-way double blind crossover study. Studies were performed at the same time of day on each visit and separated by 1 week. An 18-standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa, under local anaesthetic using aseptic technique. Subjects remained seated for 20 min to establish baseline haemodynamic conditions, and underwent systemic administration of uric acid 500 mg in 250 ml vehicle, or 250 ml vehicle alone over 20 min via the non-dominant forearm cannula. Subjects performed lower limb exercise using an upright electronically-braked ergometric cycle (Ergometry System 380B, Siemens-Elema, Sweden) for 20 min. Pedalling rate was sustained at 70 ± 10 Hz to maintain a constant workload of 80 W, equivalent to a moderately intense riding speed of 3.8 ms-1 (8.5 mph) [296]. Exercise was followed by a 20 min recovery period during which subjects rested seated.

Heart rate, blood pressure and cardiac index measurements were recorded at baseline, and at 5 min intervals up to 60 min after the start of the infusion. A 5 ml venous blood sample was collected in serum gel tubes via the non-infused forearm cannula, at baseline and 20, 40, and 60 min after the start of infusion, for measurement of serum uric acid concentration and antioxidant capacity using the 'Total Antioxidant Status' assay. Additional 5 ml samples were collected in potassium-EDTA tubes (Sarstedt Ltd., Leicester, UK) at baseline and 60 min after the start of infusion for determination of plasma 8-*iso*-$PGF_{2\alpha}$ concentrations. Blood samples were centrifuged at 1000 *g* for 10 minutes at 4°C, decanted immediately, and serum and plasma were stored at -40°C until assays were performed.

11.3 Results

Table 28. Baseline characteristics of the study population

Characteristic	Mean ± SD	Characteristic	Mean ± SD
Number/ men	20/ 10	Body mass index (kg/m^2)	23.0 ± 3.5
Age (y)	23 ± 3	Serum creatinine (μmol/l)	72 ± 10
Height (m)	1.72 ± 0.34	Serum cholesterol (mmol/l)	4.0 ± 2.5
Weight (kg)	71.5	Serum uric acid (μmol/l)	293 ± 52

11.3.1 Uric acid concentrations

Administration of vehicle and uric acid caused mean ± SEM increases in serum uric acid concentrations of –9 ± 2 and 194 ± 8 μmol/l respectively ($p < 0.001$).

Table 29. Mean ± SEM serum uric acid concentrations (μmol/l) at baseline, after infusion of uric acid 500 mg in vehicle or vehicle alone, after exercise and after recovery. $^{*}p < 0.001$ compared to baseline.

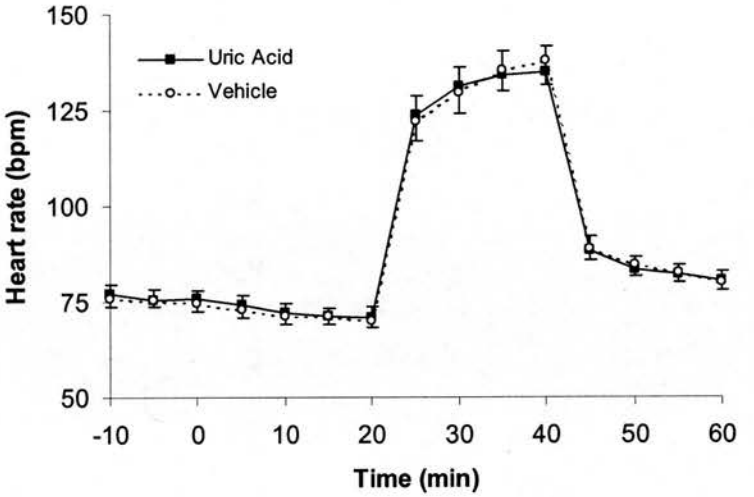
	Baseline	**Post-infusion**	**Post-exercise**	**Post-recovery**
	0 min	20 min	40 min	60 min
Vehicle	294 ± 19	286 ± 18	296 ± 19	293 ± 18
Uric acid	293 ± 16	487 ± 16*	458 ± 15*	429 ± 14*

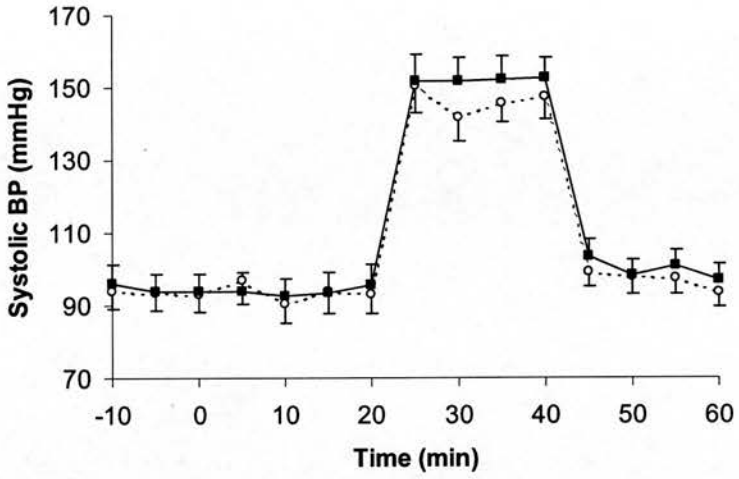
11.3.2 Haemodynamic variables

Intense physical exercise caused significant increases in heart rate, systolic blood pressure, diastolic blood pressure and cardiac index, and a reduction in systemic vascular resistance index. Uric acid administration did not influence haemodynamic variables at rest nor the haemodynamic response to exercise.

Table 30. Mean ± SEM haemodynamic variables at baseline, after infusion of uric acid or vehicle, after exercise and after recovery. $^{*}p < 0.005$ compared to baseline.

		Baseline	**Post-infusion**	**Post-exercise**	**Post-recovery**
		0 min	20 min	40 min	60 min
Heart rate (bpm)	Vehicle	75 ± 2	73 ± 2	138 ± 6*	80 ± 2
	Uric acid	76 ± 3	74 ± 2	135 ± 7*	80 ± 3
Systolic BP	Vehicle	93 ± 5	97 ± 6	147 ± 6*	94 ± 4
(mm Hg)	Uric acid	94 ± 5	94 ± 5	153 ± 6*	97 ± 5
Diastolic BP	Vehicle	66 ± 2	67 ± 3	101 ± 5*	69 ± 2
(mm Hg)	Uric acid	68 ± 2	66 ± 4	103 ± 6*	68 ± 2
Cardiac index	Vehicle	3.9 ± 0.2	3.8 ± 0.2	9.7 ± 0.8*	3.8 ± 0.2
($l/min.m^2$)	Uric acid	3.9 ± 0.2	3.7 ± 0.1	9.4 ± 0.6*	4.0 ± 0.2
Sys. vascular	Vehicle	22.0 ± 2.0	23.2 ± 1.7	15.9 ± 2.4*	22.0 ± 1.7
resistance index	Uric acid	22.4 ± 1.9	23.6 ± 1.6	14.5 ± 1.2*	23.0 ± 2.0





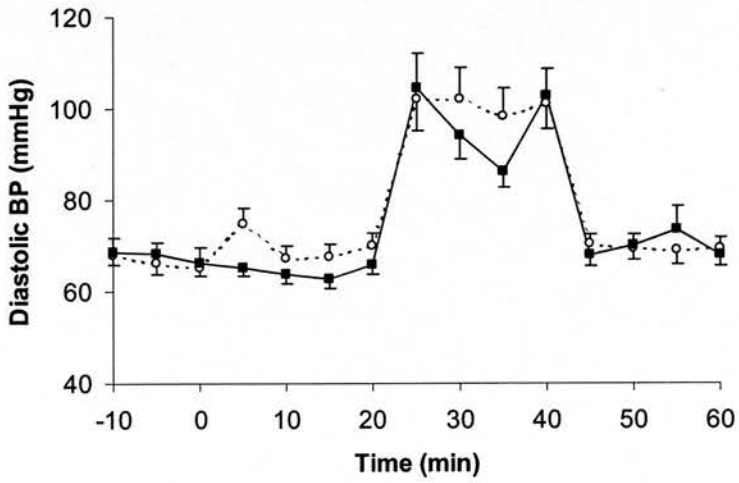


Figure 16, continued overleaf.

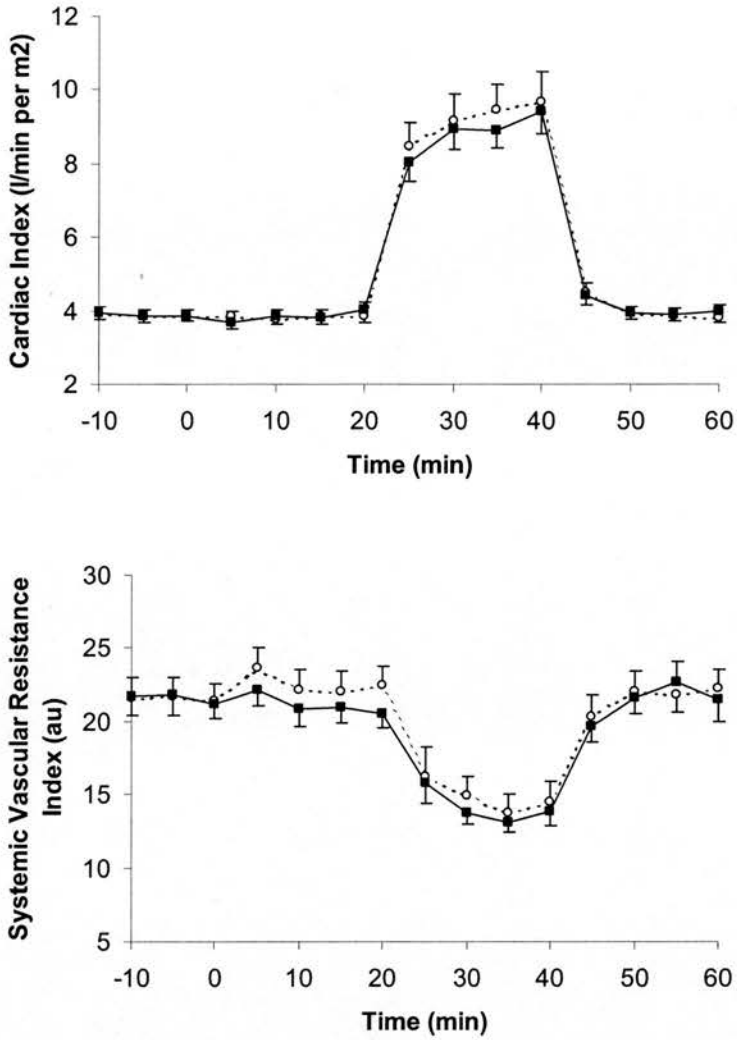


Figure 16. Haemodynamic variables during infusion of uric acid 500 mg or vehicle (0-20 min), exercise (20-40 min) and recovery (40-60 min) shown as mean ± SEM.

11.3.3 Serum antioxidant capacity

Administration of vehicle and uric acid caused respective changes in serum antioxidant capacity from baseline of -22 ± 23 and 118 ± 18 μmol/l ($p < 0.001$ between infusions).

Table 31. Mean ± SEM serum antioxidant capacity (μmol/l) at baseline, after uric acid or vehicle infusion, exercise and recovery. $^{*}p < 0.005$ compared to baseline.

	Baseline	**Post-infusion**	**Post-exercise**	**Post-recovery**
	0 min	20 min	40 min	60 min
Vehicle	1815 ± 29	1769 ± 46	1817 ± 27	1796 ± 32
Uric acid	1786 ± 39	1899 ± 45**	1937 ± 44*	1895 ± 47*

11.3.4 Plasma 8-isoprostaglandin $F_{2\alpha}$ concentrations

Intense physical exercise caused a significant increase in plasma 8-*iso*-$PGF_{2\alpha}$ concentrations from 35.0 ± 4.7 pg/ml to 45.6 ± 6.7 pg/ml immediately after exercise ($p < 0.05$), and 55.7 ± 7.1 pg/ml after recovery ($p < 0.01$), following vehicle administration. After uric acid administration, 8-*iso*-$PGF_{2\alpha}$ concentrations (37.1 ± 5.4 pg/ml) did not increase after exercise (31.6 ± 5.5 pg/ml) or recovery (37.9 ± 6.6 pg/ml). Plasma 8-*iso*-$PGF_{2\alpha}$ concentrations after vehicle and uric acid administration, expressed as change from baseline, were 10.6 ± 5.5 and -5.5 ± 6.2 pg/ml ($p < 0.05$) respectively after exercise, and 20.7 ± 6.5 and 0.8 ± 7.7 ($p < 0.01$) respectively after recovery. Uric acid administration significantly reduced isoprostane concentrations ($p = 0.02$) and changes from baseline ($p < 0.01$) by two-way ANOVA.

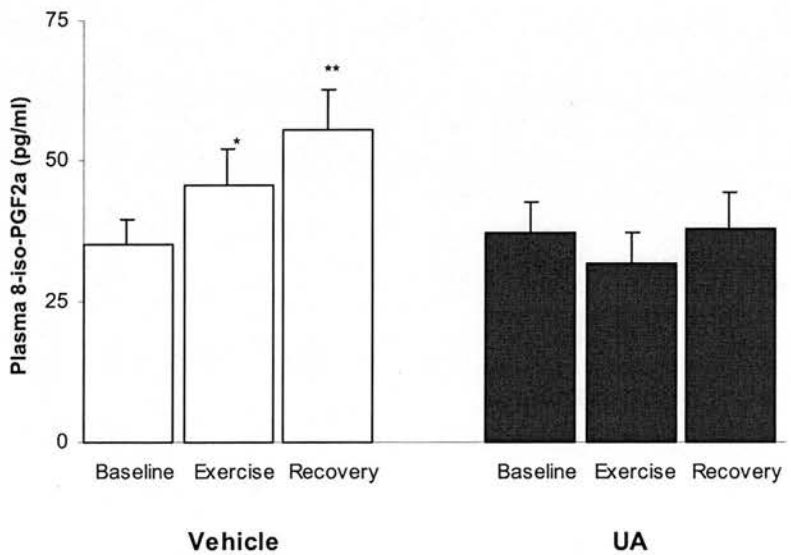


Figure 17. Plasma 8-*iso*-$PGF_{2\alpha}$ concentrations at baseline, post-exercise and post-recovery after systemic administration of uric acid 500 mg or vehicle alone.

11.4 Discussion

Resting plasma free 8-*iso*-$PGF_{2\alpha}$ concentrations were consistent with those previously reported in young, healthy people free from major cardiovascular risk factors that could potentially exert independent effects on oxidative stress. Previously reported in healthy subjects [297]. The chosen exercise intensity and duration caused a significant haemodynamic response in this group, who were unaccustomed to regular strenuous exercise. Furthermore, single bouts of acute intense exercise caused oxidative stress in the study population, reflected by increased plasma 8-*iso*-$PGF_{2\alpha}$ concentrations immediately after exercise and after recovery. The magnitude of the rise in plasma isoprostane concentrations was similar to previous observations in healthy subjects and trained athletes after acute exercise [298].

Administration of uric acid 500 mg achieved a substantial increase in circulating uric acid concentrations, associated with increased serum free radical-scavenging capacity as indicated by the 'Total Antioxidant Status' assay. Moreover, uric acid administration attenuated the exercise-induced increase in plasma 8-*iso*-$PGF_{2\alpha}$ concentrations. It is likely that this effect was mediated by increased ability to counter excess free radical activity, conferred by the antioxidant properties of uric acid. These findings indicate that high circulating uric acid concentrations are able to prevent oxidative stress *in vivo* during intense physical exercise, and raise the possibility that uric acid could protect against oxidative stress in other situations.

The lack of effect of uric acid administration on resting and exercising haemodynamic is consistent with observations described earlier in this thesis, and support the earlier observations that high uric acid concentrations do not directly influence vascular tone or systemic blood pressure, at least in the acute setting. A potential limitation is that the validity of the Omron HEM-705CP device has not been established in exercising subjects. It is accurate for measuring blood pressure up to 160/ 100 mmHg in resting subjects, and has been validated according to criteria of the British Hypertension Society and Association for the Advancement of Medical Instrumentation [267].

Chapter 12.

Intravenous Administration of Uric Acid and Endothelial Function, Large Arterial Stiffness and Systemic Haemodynamics in Regular Smokers and Patients with Type 1 Diabetes Mellitus

12.1 Introduction

Endothelial dysfunction is characteristically impaired in regular cigarette smokers and patients with type 1 diabetes mellitus [25, 28]. Endothelial dysfunction in the setting of major cardiovascular risk factors appears to be a consequence of increased free radical activity, in the bloodstream or arterial intima. Endothelial function can be restored by administration of supplementary natural antioxidants, including ascorbic acid and tocopherol [299, 300]. The potential role of uric acid as a protective antioxidant has not been examined in this setting.

Regular smokers and patients with type 1 diabetes have low serum uric acid concentrations, which could increase susceptibility to oxidative damage by the excessive free radical production characteristically found in both groups. The purpose of the present study was to investigate the hypothesis that administration of uric acid or vitamin C, both powerful aqueous antioxidants, might improve vascular function in regular smokers and patients with type 1 diabetes mellitus, groups characteristically exposed to increased oxidative stress and endothelial dysfunction.

12.2 Methods

Eight regular smokers, 8 patients with type 1 diabetes, and 8 age-matched healthy controls were recruited to a 4-way randomised placebo-controlled crossover study that allowed at least one week between study visits. An 18-standard gauge cannula was inserted into a vein in the antecubital fossa of each arm, using aseptic technique and local anaesthetic. The non-dominant forearm cannula allowed infusion of uric acid 1000 mg in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, 500 ml vehicle alone, 1000 mg vitamin C in 500 ml 0.9% saline, or 500 ml saline alone, over 1 h. The brachial artery of the non-dominant forearm was cannulated to allow intra-arterial administration of acetylcholine, sodium nitroprusside and L-NMMA.

Venous blood (5 ml) was drawn from the non-infused forearm cannula, before and after systemic administration for measurement of serum uric acid concentration and antioxidant capacity. Heart rate, blood pressure, cardiac index, augmentation index

and baroreflex sensitivity by sequence and spectral analysis methods were determined before and after systemic infusion.

12.3 Results

Table 32. Baseline characteristics of the study population at screening, mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.001$ compared to controls.

	Regular smokers	**Patients with type 1 diabetes**	**Healthy Controls**
Number (men)	8 (8)	8 (8)	8 (8)
Age (y)	30 ± 2	30 ± 2	30 ± 2
Systolic BP (mmHg)	120 ± 2*	130 ± 2**	111 ± 2
Diastolic BP (mmHg)	70 ± 1**	77 ± 2**	64 ± 2
Heart rate (bpm)	60 ± 3	70 ± 2**	60 ± 1
Body mass index (kg/m^2)	26 ± 1	24 ± 1	25 ± 1
Creatinine (μmol/l)	79 ± 2	63 ± 5*	82 ± 3
Glucose (mmol/l)	4.7 ± 0.1	8.5 ± 1.1**	4.6 ± 0.1
Cholesterol (mmol/l)	4.2 ± 0.1*	4.2 ± 0.4*	3.6 ± 0.2

Plasma glucose concentrations were significantly higher in patients with diabetes compared to healthy subjects ($p < 0.001$) and smokers ($p < 0.001$), but did not vary significantly between study visits.

12.3.1 Serum uric acid concentrations

Baseline serum uric acid concentrations, across all study visits, were significantly lower among regular smokers (315 ± 11 μmol/l, $p < 0.05$) and patients with type 1 diabetes (245 ± 18 μmol/l, $p < 0.001$) than in healthy controls (336 ± 11 μmol/l). Uric acid administration significantly increased serum concentrations by 286 ± 17, 270 ± 20, and 262 ± 18 μmol/l in each group respectively.

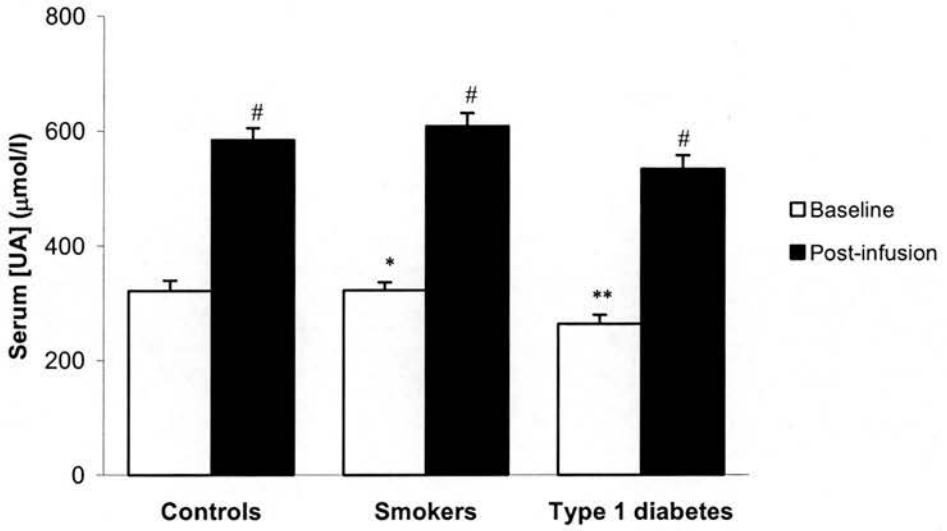


Figure 18: Serum uric acid concentrations before and after administration uric acid 1000 mg in regular smokers, patients with type 1 diabetes, and healthy controls. $^{*}p < 0.05$, $^{*}p < 0.005$ compared to controls, $^{\#}p < 0.001$ compared to baseline.

12.3.2 Serum antioxidant capacity

Antioxidant capacity was significantly lower in regular smokers and patients with type 1 diabetes than healthy controls. Administration of uric acid and vitamin C increased serum antioxidant capacity in all groups, but saline and vehicle did not.

Table 33. Mean ± SEM serum antioxidant capacity in smokers, patients with type 1 diabetes and controls (µmol/l Trolox eq.), before and after administration of uric acid or vitamin C. $^{*}p < 0.05$ versus controls, $^{\ddagger}p < 0.05$, $^{\ddagger\ddagger}p < 0.01$ versus baseline.

		Regular Smokers	**Patients with type 1 diabetes**	**Healthy Controls**
Vitamin C	Before	$1203 \pm 46^{*}$	$1089 \pm 46^{*}$	1404 ± 57
administration	After	$1316 \pm 50^{\ddagger}$	$1240 \pm 48^{\ddagger}$	$1532 \pm 66^{\ddagger}$
	Change	$120 \pm 12^{\ddagger\ddagger}$	$148 \pm 17^{\ddagger\ddagger}$	$131 \pm 13^{\ddagger\ddagger}$
Uric acid	Before	1224 ± 54	$1106 \pm 48^{*}$	1341 ± 60
administration	After	$1402 \pm 66^{\ddagger}$	$1349 \pm 52^{\ddagger\ddagger}$	$1578 \pm 67^{\ddagger\ddagger}$
	Change	$203 \pm 22^{\ddagger\ddagger}$	$221 \pm 19^{\ddagger\ddagger}$	$235 \pm 30^{\ddagger\ddagger}$

12.3.3 Haemodynamic variables

Administration of neither uric acid, vehicle, vitamin C nor saline had any significant effects on heart rate, blood pressure, cardiac index or systemic vascular resistance index in regular smokers, patients with type 1 diabetes and healthy controls.

Table 34. Mean ± SEM haemodynamic variables in regular smokers, patients with type 1 diabetes and healthy controls subjects, before and immediately after systemic administration of 0.9% saline, vehicle, uric acid 1000 mg or vitamin C 1000 mg.

		Regular smokers	**Patients with type 1 diabetes**	**Healthy controls**
Saline administration				
Heart rate (bpm)	Before	71 ± 1	72 ± 6	59 ± 5
	After	67 ± 4	76 ± 6	56 ± 4
Systolic BP (mmHg)	Before	125 ± 5	124 ± 3	115 ± 4
	After	129 ± 5	132 ± 9	117 ± 5
Cardiac index (l/m^2)	Before	3.0 ± 0.4	3.6 ± 0.3	3.2 ± 0.3
	After	3.1 ± 0.2	3.8 ± 0.5	3.1 ± 0.2
SVRI (au)	Before	32.6 ± 4.9	26.0 ± 1.4	27.3 ± 2.5
	After	34.6 ± 4.6	27.7 ± 2.7	28.5 ± 2.5
Vehicle administration				
Heart rate (bpm)	Before	71 ± 2	71 ± 7	58 ± 3
	After	71 ± 4	67 ± 7	56 ± 5
Systolic BP (mmHg)	Before	121 ± 4	133 ± 7	119 ± 5
	After	125 ± 3	134 ± 7	113 ± 7
Cardiac index (l/m^2)	Before	3.6 ± 0.2	3.5 ± 0.4	3.4 ± 0.8
	After	3.1 ± 0.2	3.7 ± 0.3	2.8 ± 0.3
SVRI (au)	Before	24.8 ± 1.1	25.2 ± 4.8	29.7 ± 3.9
	After	30.3 ± 1.5	27.3 ± 2.6	31.7 ± 2.4
Uric acid administration				
Heart rate (bpm)	Before	71 ± 4	68 ± 5	63 ± 3
	After	68 ± 6	70 ± 7	59 ± 2

Table 34 continued overleaf.

Table 34 continued.

		Regular smokers	Patients with type 1 diabetes	Healthy controls
Uric acid administration				
Systolic BP (mmHg)	Before	124 ± 3	131 ± 5	119 ± 4
	After	127 ± 6	138 ± 4	118 ± 3
Cardiac index (l/m^2)	Before	3.0 ± 0.5	3.6 ± 0.3	3.0 ± 0.3
	After	3.0 ± 0.4	3.3 ± 0.3	2.9 ± 0.3
SVRI (au)	Before	32.9 ± 4.7	26.7 ± 2.3	28.7 ± 2.5
	After	33.4 ± 4.8	30.9 ± 1.9	30.8 ± 2.3
Vitamin C administration				
Heart rate (bpm)	Before	72 ± 4	70 ± 5	60 ± 4
	After	69 ± 3	71 ± 6	58 ± 4
Systolic BP (mmHg)	Before	123 ± 4	130 ± 5	118 ± 4
	After	124 ± 4	133 ± 6	116 ± 3
Cardiac index (l/m^2)	Before	3.3 ± 0.3	3.5 ± 0.4	3.2 ± 0.5
	After	3.2 ± 0.4	3.6 ± 0.3	2.9 ± 0.3
SVRI (au)	Before	29.2 ± 2.8	25.8 ± 3.2	28.5 ± 2.2
	After	31.4 ± 3.1	27.2 ± 2.7	30.4 ± 2.0

12.3.4 Augmentation index

Baseline augmentation index values were significantly higher in regular smokers ($p < 0.001$) and patients with type 1 diabetes ($p < 0.001$) than healthy controls.

Vitamin C was associated with a non-significant trend towards reduced augmentation index in patients with type 1 diabetes ($p = 0.062$), and had no effect in regular smokers and healthy subjects. Neither uric acid, vehicle nor saline caused any significant effects on augmentation index in smokers, patients with type 1 diabetes or healthy controls.

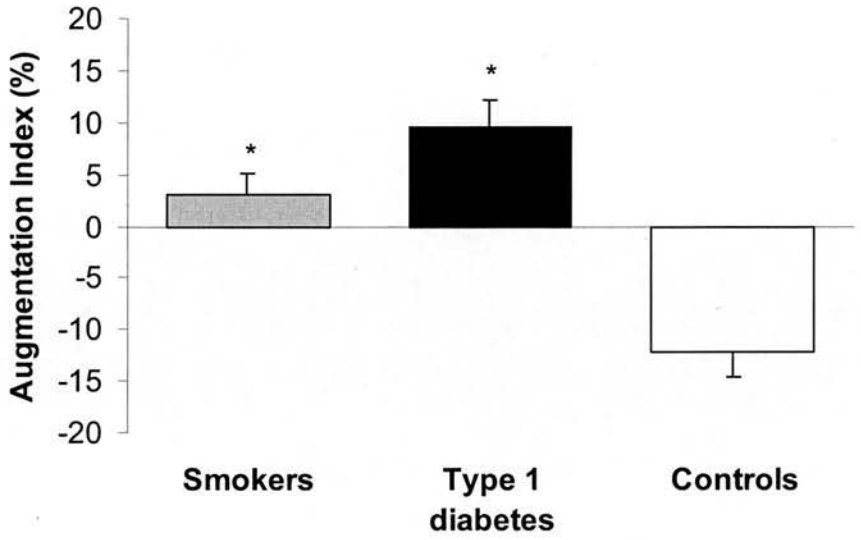


Figure 19. Mean ± SEM baseline augmentation index (%) in regular smokers, patients with type 1 diabetes and healthy controls. $^{*}p < 0.001$ compared to controls.

Table 35. Mean ± SEM augmentation index (%)in smokers, patients with type 1 diabetes and controls, before and after administration of 0.9% saline, vehicle, uric acid 1000 mg or vitamin C 1000 mg. $^{*}p < 0.005$, $^{**}p < 0.001$ compared to controls.

		Regular smokers	**Patients with type 1 diabetes**	**Healthy subjects**
Saline administration	Before	$5.3 \pm 3.6^{**}$	$9.1 \pm 4.9^{**}$	-12.3 ± 4.7
	After	6.0 ± 4.8	10.7 ± 4.5	-11.6 ± 4.5
Vehicle administration	Before	$5.3 \pm 3.2^{**}$	$9.6 \pm 4.5^{**}$	-12.9 ± 4.6
	After	5.7 ± 4.7	14.2 ± 4.7	-10.5 ± 5.0
Uric acid administration	Before	$4.8 \pm 3.5^{*}$	$9.9 \pm 4.5^{**}$	-11.2 ± 4.7
	After	7.0 ± 4.1	11.9 ± 4.8	-10.8 ± 4.6
Vitamin C administration	Before	$5.2 \pm 3.4^{**}$	$9.8 \pm 4.7^{**}$	-11.9 ± 4.5
	After	5.6 ± 4.4	6.3 ± 4.4	-12.2 ± 4.6

12.3.5 Baroreflex sensitivity

Baroreflex sensitivity, determined by both sequence analysis and spectral analysis methods, was significantly impaired in regular smokers and patients with type 1 diabetes mellitus compared to healthy controls.

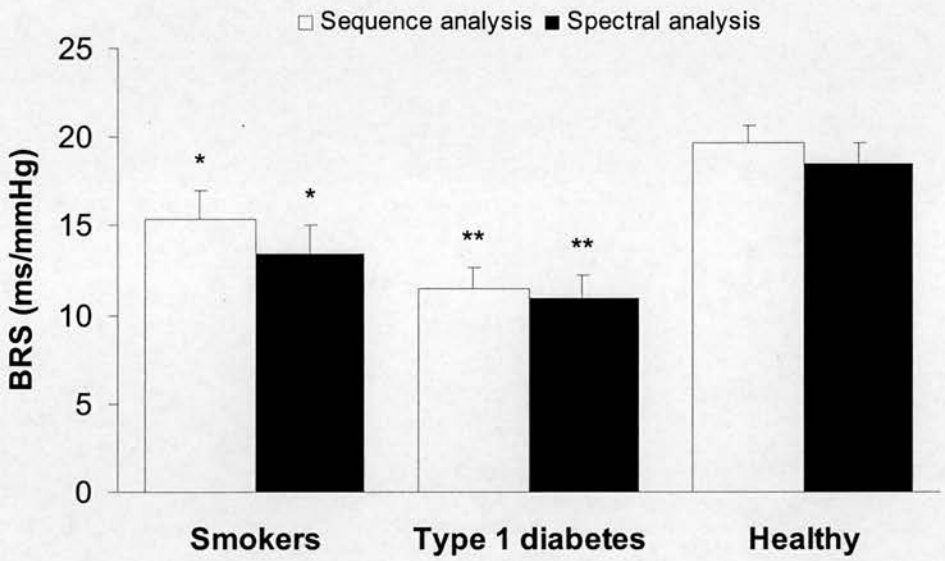


Figure 20. Mean ± SEM baroreflex sensitivity in smokers, patients with type 1 diabetes and healthy controls, determined by sequence and spectral analysis methods.

Systemic administration of neither 0.9% saline, vehicle, uric acid 1000 mg nor vitamin C 1000 mg caused any significant effect on baroreceptor reflex sensitivity from baseline in smokers, patients with type 1 diabetes or controls (shown overleaf).

12.3.6 Endothelial function

Blood flow responses to acetylcholine and sodium nitroprusside after systemic saline administration were used to represent baseline vascular function in each group. Acetylcholine responses were lower in smokers ($p < 0.005$) and patients with type 1 diabetes ($p < 0.001$) than controls. Uric acid and vitamin C increased acetylcholine responses in smokers ($p < 0.05$ both) and patients with diabetes ($p < 0.01$ both), but not those to sodium nitroprusside. Vitamin C improved L-NMMA responses in smokers and patients with diabetes ($p < 0.05$ both). Neither uric acid nor vitamin C had any effect on blood flow responses in healthy subjects (see Figures 21 to 24).

Table 36. Mean ± SEM baroreflex sensitivity (ms/mmHg) by sequence and spectral analysis before and after administration of saline, vehicle, uric acid or vitamin C.

		Regular smokers	Type 1 diabetes	Healthy controls
Saline administration				
Sequence analysis	Before	14.6 ± 2.2	10.5 ± 2.0	18.4 ± 3.3
	After	13.1 ± 1.6	11.2 ± 2.2	20.9 ± 2.5
Spectral analysis	Before	13.6 ± 2.9	9.1 ± 2.0	17.7 ± 2.5
	After	12.7 ± 2.4	10.0 ± 2.3	17.5 ± 3.2
Vehicle administration				
Sequence analysis	Before	13.5 ± 1.9	11.7 ± 2.2	22.2 ± 3.6
	After	12.2 ± 2.0	11.7 ± 2.2	19.0 ± 2.5
Spectral analysis	Before	11.1 ± 1.5	11.4 ± 2.5	20.9 ± 4.7
	After	11.0 ± 2.0	10.4 ± 2.3	16.6 ± 2.8
Uric acid administration				
Sequence analysis	Before	16.1 ± 2.8	11.4 ± 2.1	21.7 ± 3.4
	After	14.5 ± 2.5	12.3 ± 1.5	19.9 ± 2.8
Spectral analysis	Before	12.8 ± 2.7	10.4 ± 2.1	20.3 ± 3.4
	After	12.3 ± 2.7	10.4 ± 1.7	18.4 ± 3.1
Vitamin C administration				
Sequence analysis	Before	14.8 ± 2.2	10.8 ± 2.0	19.8 ± 3.1
	After	14.9 ± 2.6	11.2 ± 1.9	20.2 ± 3.2
Spectral analysis	Before	15.8 ± 2.3	10.6 ± 1.8	18.9 ± 4.1
	After	16.1 ± 2.4	10.8 ± 2.2	20.5 ± 3.8

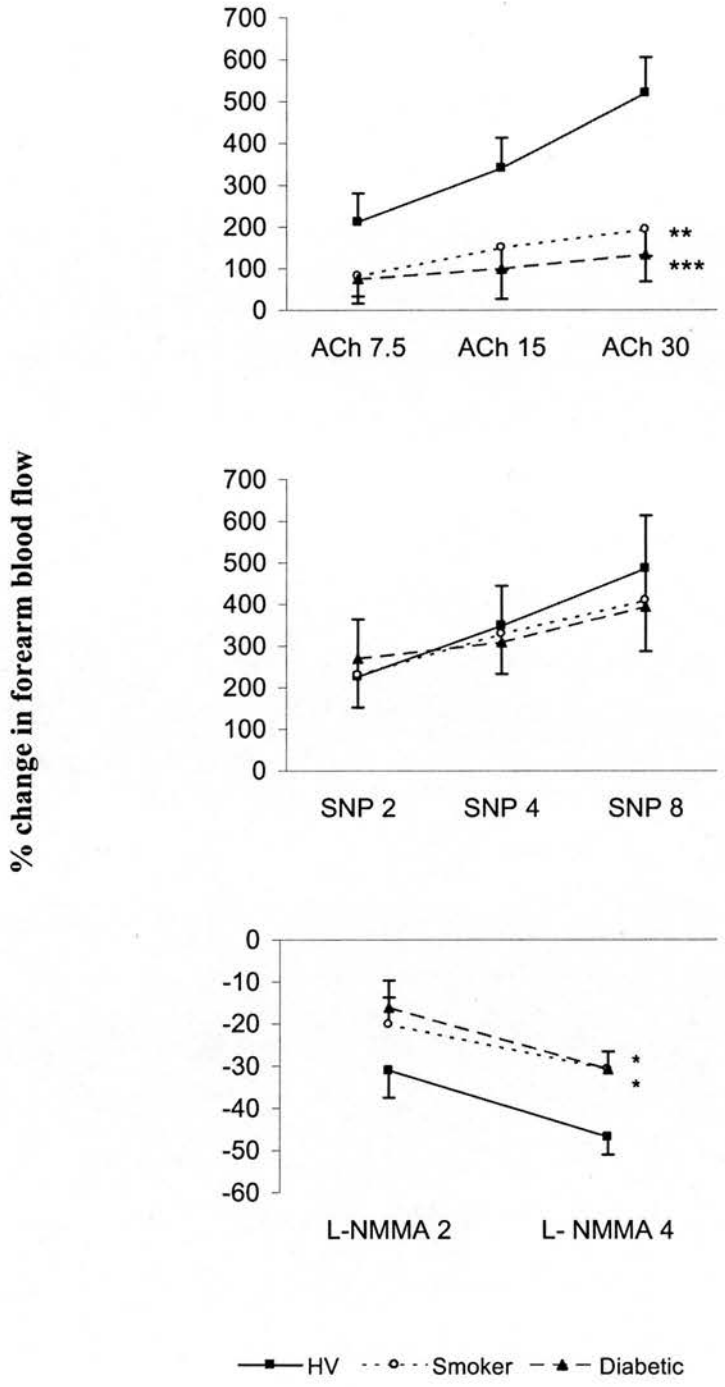


Figure 21. Forearm blood flow responses to intra-brachial acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min in regular smokers, patients with type 1 diabetes and healthy controls. $^{*}p < 0.05$, $^{**}p < 0.001$ compared to controls by ANOVA.

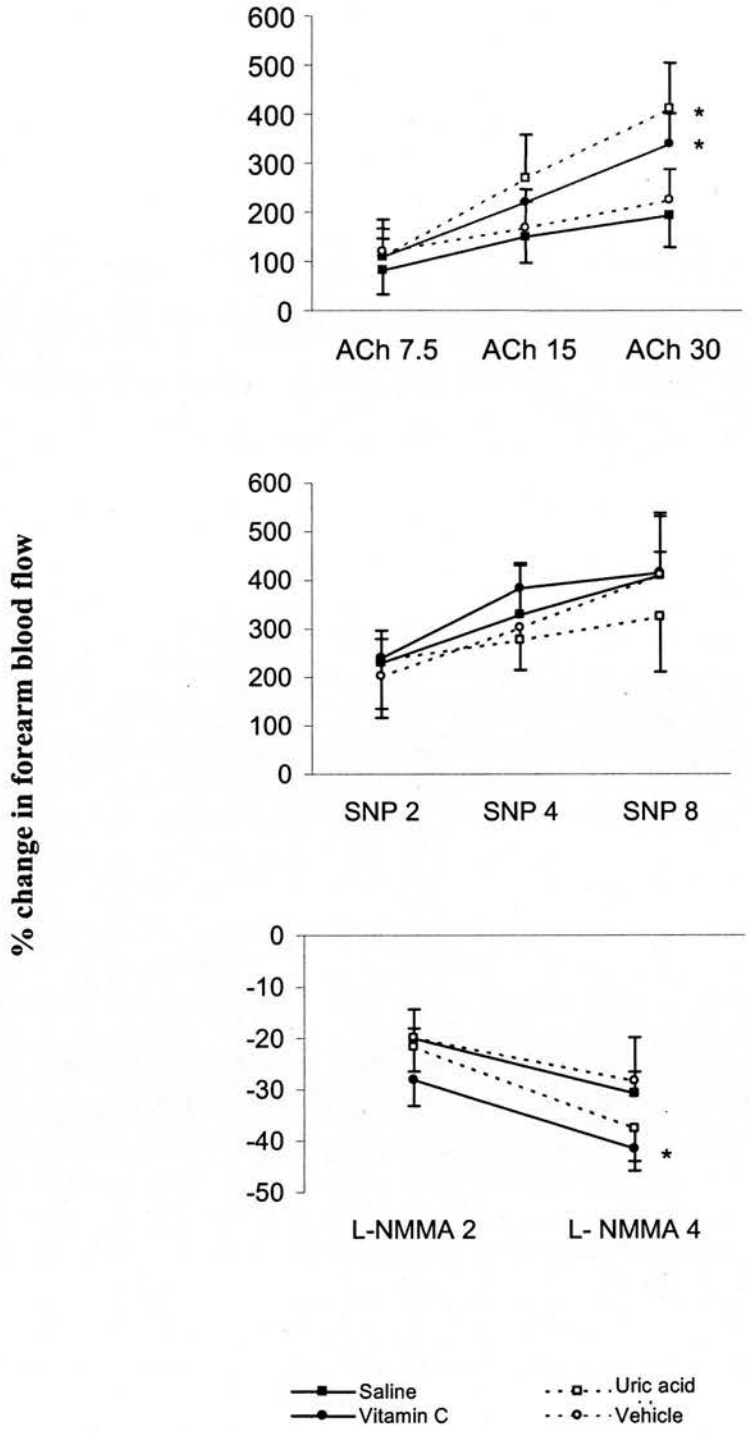


Figure 22. Forearm blood flow responses to intra-brachial acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min after i.v. uric acid 1000 mg, vitamin C 1000 mg, vehicle, or 0.9% saline in regular smokers. *$p < 0.05$, **$p < 0.001$ compared to saline by ANOVA.

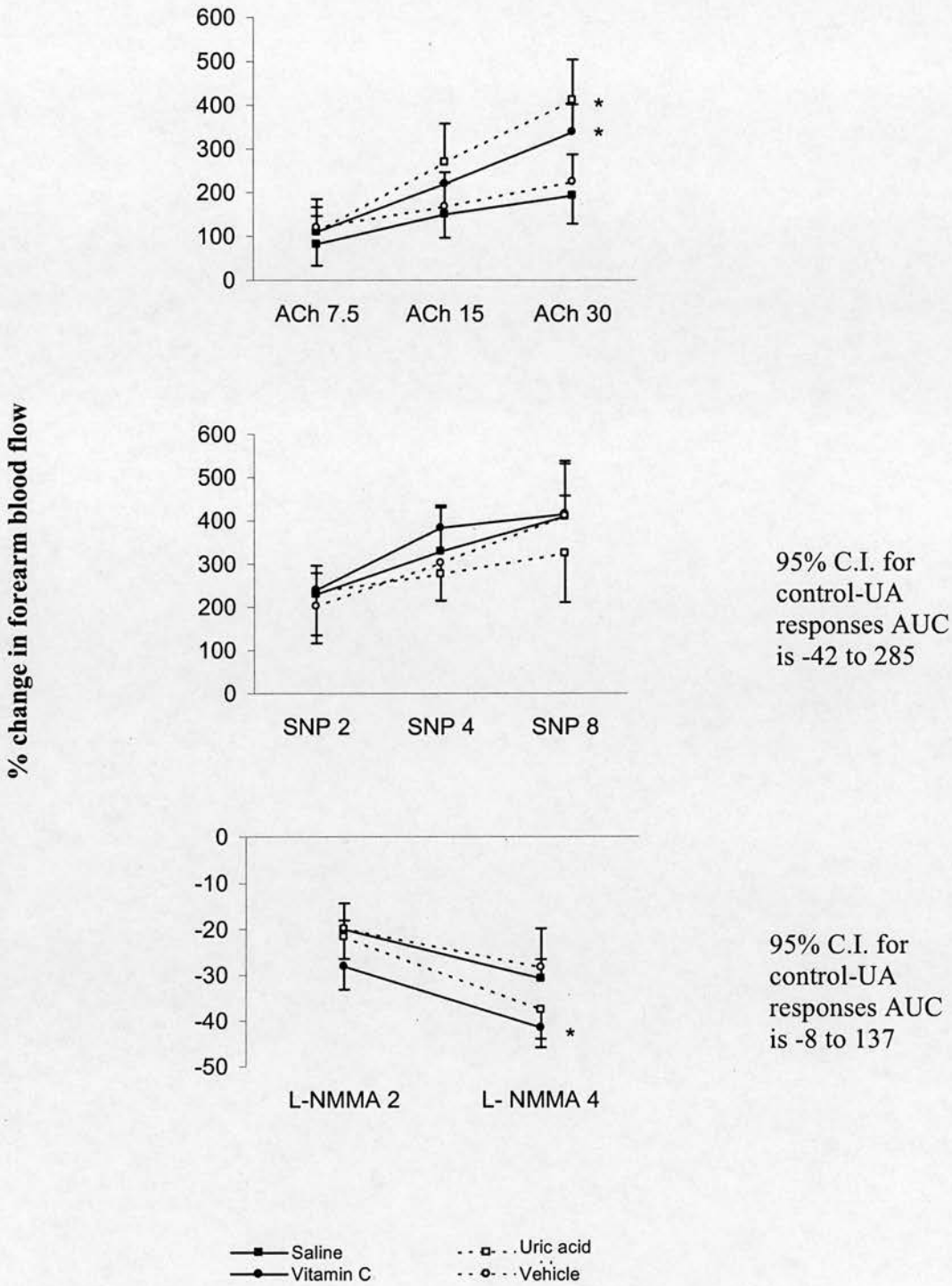


Figure 23. Forearm blood flow responses to intra-brachial acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min after i.v. uric acid 1000 mg, vitamin C 1000 mg, vehicle, or 0.9% saline in patients with type 1 diabetes. *$p < 0.05$, **$p < 0.001$ compared to saline by ANOVA.

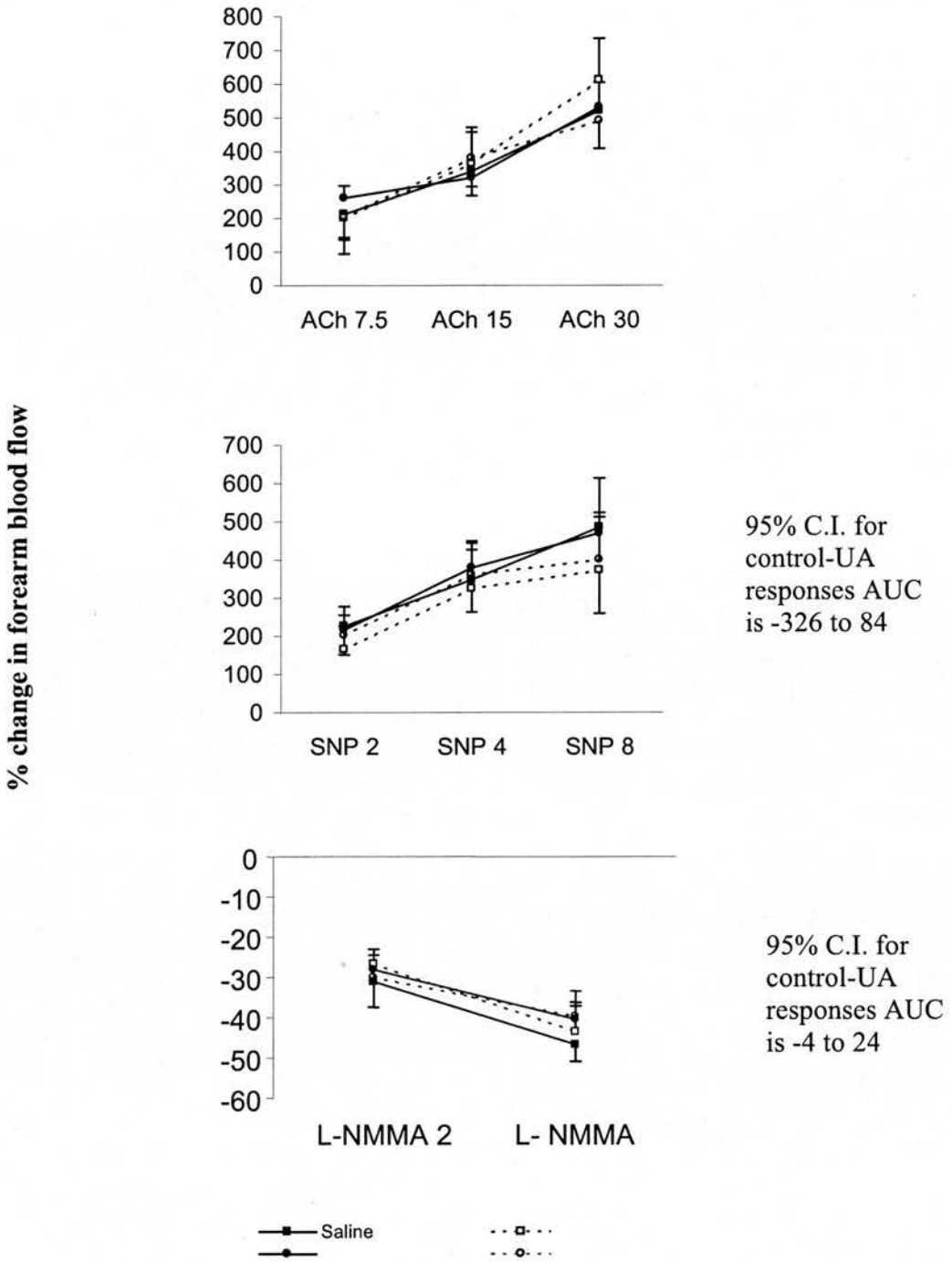


Figure 24. Mean ± SEM forearm blood flow responses to intra-brachial acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min after i.v. uric acid 1000 mg, vitamin C 1000 mg, vehicle, or 0.9% saline in healthy controls.

12.4 Discussion

Consistent with previous studies, forearm blood flow responses to intra-brachial acetylcholine were significantly impaired in regular smokers and patients with type 1 diabetes, indicating impaired endothelium-dependent vasodilator responses in these groups. Responses to L-NMMA in smokers and patients with diabetes were impaired, indicating reduced constitutive nitric oxide bioavailability in these groups, consistent with previous reports. There were no differences in responses to sodium nitroprusside between groups, indicating that endothelium-independent vascular responses to nitric oxide-mediated stimuli are intact in regular smokers and patients with type 1 diabetes. These observations indicate that uric acid and vitamin C allowed restoration of dynamic endothelium-dependent nitric oxide bioavailability.

Baseline uric acid concentrations and serum antioxidant capacity were low in regular smokers and patients with type 1 diabetes, consistent with previous reports. This may be because of more rapid consumption in the presence of increased free radical activity. Endothelium-derived nitric oxide is subjected to abnormally rapid degradation and reduced bioavailability in the setting of oxidative stress. Systemic administration of vitamin C and uric acid significantly enhanced circulating antioxidant defences. This suggests that they might protect nitric oxide against oxidative, and thereby explains the observed improvement in endothelial function. This hypothesis is supported by a lack of influence of uric acid and vitamin C on responses in healthy controls not exposed to oxidative stress and with normal antioxidant defences. The observed effects of uric acid on endothelial function are consistent with findings in a perfused guinea pig heart model, where addition of uric acid in physiological concentrations allowed restoration of acetylcholine-mediated coronary vasodilator responses in the presence of oxidative stress [301].

Vasoconstriction to L-NMMA is a marker of basal nitric oxide production, and was significantly lower in regular smokers and patients with type 1 diabetes. Only vitamin C improved L-NMMA responses in smokers and patients with diabetes. These data indicate that vitamin C but not uric acid was capable of improving basal vascular nitric oxide availability, and suggest different mechanisms might underlie

the effects of each. However, differences between basal and stimulated endothelium-dependent nitric oxide bioavailability have previously been observed in the presence of other risk factors including regular smoking [302], hypercholesterolaemia [303] and hyperhomocysteinaemia [304]. Stimulated nitric oxide dependent responses may be a more sensitive marker of endothelial function than basal nitric oxide activity.

Other mechanisms might account for differences between the effects of vitamin C and uric acid. For example, vitamin C is able to diffuse passively across endothelial cell membranes, whereas uric acid depends on active carrier-mediated transport into endothelial cells, and intracellular concentrations are lower than those found in the circulation due to active cellular efflux. So it is possible that despite significant increases in circulating uric acid concentration, there might be less impact on intracellular antioxidant activity.

A weakness of the present findings is that the small numbers of study subjects and comparatively wide confidence intervals do not fully exclude the possibility of an effect of uric acid administration on L-NMMA responses in smokers and patients with diabetes. A limitation of this study is the lack of circulating markers of oxidative stress, and the mechanisms underlying the present findings require further exploration. However, both regular smokers and patients with type 1 diabetes have consistently been shown to be exposed to greater oxidant activity than healthy individuals, and have higher circulating markers of vascular oxidative damage.

An additional limitation is that serum cholesterol and blood pressure appear to be potentially important confounding factors because both were higher in regular smokers and patients with type 1 diabetes than controls. Endothelial dysfunction has been consistently reported in regular smokers and patients with type 1 diabetes, irrespective of lipid profile. Although retrospective analyses did not identify any relationship between blood pressure and blood flow responses, vitamin C can reverse endothelial dysfunction in patients with hypertension. Further work is required to explore whether uric acid restores endothelial function in patients with other risk factors for cardiovascular disease and endothelial dysfunction.

Chapter 13.

Intravenous Administration of Urate Oxidase and Vascular Function in Patients With Type 2 Diabetes Mellitus

13.1 Introduction

Epidemiological studies have found that patients with type 2 diabetes have higher serum uric acid concentrations than healthy people and, within this patient group, high serum uric acid concentrations are associated with increased cardiovascular risk. Clinical studies have found that blood flow responses to endothelial-dependent nitric oxide-mediated responses are blunted in patients with type 2 diabetes. Increased large arterial stiffness is a characteristic finding in patients with type 2 diabetes, presumably as a manifestation of endothelial dysfunction and loss of nitric oxide bioavailability in large arteries.

One previous study found that administration of allopurinol, to lower serum uric acid concentrations, restored endothelial function in patients with type 2 diabetes. The effect of allopurinol on endothelial function might be explained by the associated reduction in serum uric acid concentration, which would support the hypothesis that uric acid is an independent, causal cardiovascular risk factor. However, allopurinol possesses additional intrinsic antioxidant properties, and inhibits xanthine oxidase-dependent free radical liberation. Therefore, it is difficult to draw conclusions about the potential benefits that might be achieved by uric acid lowering in patients with type 2 diabetes.

The present study was constructed to directly examine the potential cardiovascular effects of reducing serum uric acid concentration in patients with type 2 diabetes, by means of urate oxidase administration. Urate oxidase is capable of lowering serum uric acid concentrations by a greater extent than typically achieved by oxypurinol or allopurinol administration, and avoids the confounding effects associated with xanthine oxidase inhibition. If uric acid is an independent causal factor in mediating endothelial dysfunction in patients with type 2 diabetes, then lowering serum uric acid concentrations might allow restoration of endothelial function.

13.1.1 Urate oxidase

Urate oxidase (E.C. 1.7.3.3) is an enzyme that catalyses the oxidation of uric acid to allantoin in virtually all other species apart from man and higher apes. Allantoin is

around 5-10 times more water soluble at tubular pH than uric acid, and is readily excreted by the kidneys [305]. Humans remain exposed to low circulating concentrations of allantoin arising from non-enzymatic oxidation, particularly in the setting of oxidative stress. A normal reference range for allantoin (expressed as a percentage of circulating uric acid concentrations) has been proposed as 2-8% in healthy subjects [306, 307].

Early pharmaceutical preparations of fungal origin had been used to successfully treat severe hyperuricaemia in patients who were allergic to allopurinol, but were associated with a high rate of anaphylactoid reactions and allergy [308]. More recently, the enzyme has been synthesised on a much wider scale using recombinant technology, and a number of highly purified preparations are now available. For example, parenteral administration of rasburicase has been shown to be effective in treating and preventing severe hyperuricaemia in the setting of tumour lysis syndrome in children and adult patients [309]. At present, it is licensed for use within a number of European countries, and available in the United Kingdom on a named patient basis only. It has been shown to cause profound reductions in circulating and urinary uric acid concentrations, thereby allows restoration of normal renal function in the setting of acute uric acid nephropathy [110]. Rasburicase is well tolerated, and principal adverse effects reported are headache (1.8%), fever (1.4%) and allergic-type reactions (0.7%) [309].

The use of rasburicase is associated with dramatic reductions in circulating serum uric acid concentrations, for example lowering concentrations from 11.3 to 0.2 g/dl (672 to 12 mmol/l) in children and from 13.1 to 0.3 g/dl (779 to 18 mmol/l) in adult patients with severe hyperuricaemia [309]. To reduce normal or modestly elevated serum uric acid concentrations might require lower doses than have conventionally been used in oncology patients. The present study was designed to identify the most appropriate dose of urate oxidase that would allow substantial lowering of serum concentrations, and yet minimise the potential risks associated with drug exposure. This would allow the potential cardiovascular effects of uric acid lowering, by means of urate oxidase administration, to be examined in subsequent studies. Urate oxidase

itself might exert a direct effect on cardiovascular function, which might be an important confounder. Therefore, an additional objective of the present study was to characterise the duration of uric acid lowering after a single systemic administration of urate oxidase. The optimum time to examine cardiovascular function in future studies would be selected as the longest possible time after urate oxidase administration at which substantially lowered uric acid concentrations persist.

13.2 Methods

13.2.1 Dose-finding study

Two healthy men aged 33 and 36 years were studied in a 5-step single ascending dose study. Both underwent systemic administration of urate oxidase 0, 0.375, 0.75, 1.125 and 1.5 mg by bolus intravenous administration during separate visits at least 1 week apart. The timing of placebo during dose escalation was randomly allocated. Venous blood was collected for serum uric acid measurement at baseline and at 0.25, 0.5, 0.75, 1, 2, 4, 12, 24, 48, 72, and 96 h after drug administration.

13.2.2 Main protocol

Eight patients with type 2 diabetes and 8 age and sex-matched healthy control subjects were recruited to a two way, randomised, placebo-controlled, crossover study. An 18-standard gauge intravenous cannula was inserted into a suitable vein of the non-dominant antecubital fossa, using local anaesthetic and aseptic technique. Subjects rested semi-supine for 20 min to establish baseline haemodynamic conditions, and underwent intravenous administration of urate oxidase (rasburicase, Sanofi-Synthelabo, Paris, France) 1.5 mg in 10 ml 0.9% saline, or saline alone, over 1 min. Augmentation index was determined by pulse wave analysis, heart rate and blood pressure were recorded in the dominant arm immediately pre-dose, and at 15 min intervals up to 60 min after drug administration.

The venous cannula was removed, and subjects were allowed to leave and asked to return the next day. At 24 h post-dose, subjects rested supine for at least 20 min, and repeat haemodynamic measurements were recorded. Subjects underwent insertion of a 27-standard wire gauge steel needle into the non-dominant forearm, using local

anaesthetic and aseptic technique, to allow intra-brachial administration of acetylcholine 7.5, 15 and 30 μg/min, sodium nitroprusside 2, 4 and 8 μg/min, and L-NMMA 2 and 4 mmol/min. 0.9% saline was infused for 30 min before vasoactive drug administration, and for 20 min between drugs to allow establishment of baseline blood flow. The order of infusion of acetylcholine and sodium nitroprusside was randomised between subjects, and kept constant between visits. Blood flow was measured in both forearms by venous occlusion plethysmography during the last 3 min of each 6 min infusion.

Forearm blood flow responses after administration of urate oxidase or placebo were compared using two-way analysis of variance and post-hoc *t* tests.

13.3 Results

The dose-finding study showed that administration of urate oxidase caused a dose and time-dependent lowering of serum uric acid concentrations. The greatest pharmacodynamic effect was observed between 12-24 h after administration of the maximum dose studied (1.5 mg). No adverse events occurred in any subject enrolled in either the dose-finding study or the main protocol.

Table 37. Mean ± SD percentage change in serum uric acid concentration after systemic urate oxidase administration. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to baseline.

	Administered dosage of urate oxidase (mg)				
Time post-dose (h)	**0**	**0.375**	**0.75**	**1.125**	**1.5**
1	0 ± 3	-10 ± 3*	-10 ± 7	-30 ± 6**	-40 ± 13**
2	-1 ± 6	-10 ± 2**	-10 ± 9	-23 ± 6**	-43 ± 3**
4	-3 ± 8	-12 ± 10	-16 ± 11*	-33 ± 15*	-70 ± 41**
12	-2 ± 4	-6 ± 4	-18 ± 12**	-39 ± 28	-79 ± 17**
24	6 ± 8	0 ± 22	-27 ± 21*	-48 ± 22**	-79 ± 8**
48	-	-	-6 ± 4	-21 ± 12*	-56 ± 12**
72	-	-	-	-2 ± 9	-19 ± 11*
96	-	-	-	0 ± 3	-3 ± 4

Table 38. Baseline characteristics of the study population, shown as mean ± SEM. $^{*}p < 0.05$, $^{**}p < 0.01$ compared to healthy controls.

Characteristic	Healthy controls	Patients with type 2 diabetes
Number/ male	10/ 6	10/ 6
Age (y)	43 ± 3	42 ± 3
Height (m)	1.75 ± 0.11	1.76 ± 0.10
Weight (kg)	96 ± 8	114 ± 21*
Body mass index (kg/m^2)	28.9 ± 4.9	36.7 ± 6.4*
Heart rate (min^{-1})	71 ± 7	73 ± 8
Systolic BP (mmHg)	123 ± 15	124 ± 17
Diastolic BP (mmHg)	76 ± 11	75 ± 10
Serum creatinine (μmol/l)	71 ± 12	77 ± 18
Serum cholesterol (mmol/l)	4.1 ± 0.8	4.0 ± 1.1
Total: HDL cholesterol ratio	4.4 ± 1.5	5.0 ± 2.7
Serum triglycerides (mmol/l)	1.5 ± 0.7	1.9 ± 1.0
Plasma glucose (mmol/l)	4.1 ± 0.9	6.8 ± 2.0**
Hb_{A1C} (%)	5.8 ± 1.9	7.7 ± 2.6
Haematocrit (ratio)	0.41 ± 0.03	0.39 ± 0.04
Serum urate (μmol/l)	268 ± 28	349 ± 54*

Patients with type 2 diabetes had higher mean plasma glucose and serum uric acid concentrations compared to controls, but these did not vary significantly between visits. Blood pressure and serum cholesterol concentrations were well matched.

13.3.1 Serum Uric Acid Concentrations

Table 39. Serum uric acid concentrations before and 24 h after systemic administration of urate oxidase 1.5 mg. $^{*}p < 0.001$ compared to baseline.

	Healthy controls		Patients with Type 2 diabetes	
	Saline	Urate oxidase	Saline	Urate oxidase
Pre-dose	264 ± 28	276 ± 33	344 ± 58	350 ± 45
24 h	249 ± 31	102 ± 14*	328 ± 57	118 ± 39*

13.3.2 Large arterial stiffness and systemic haemodynamics

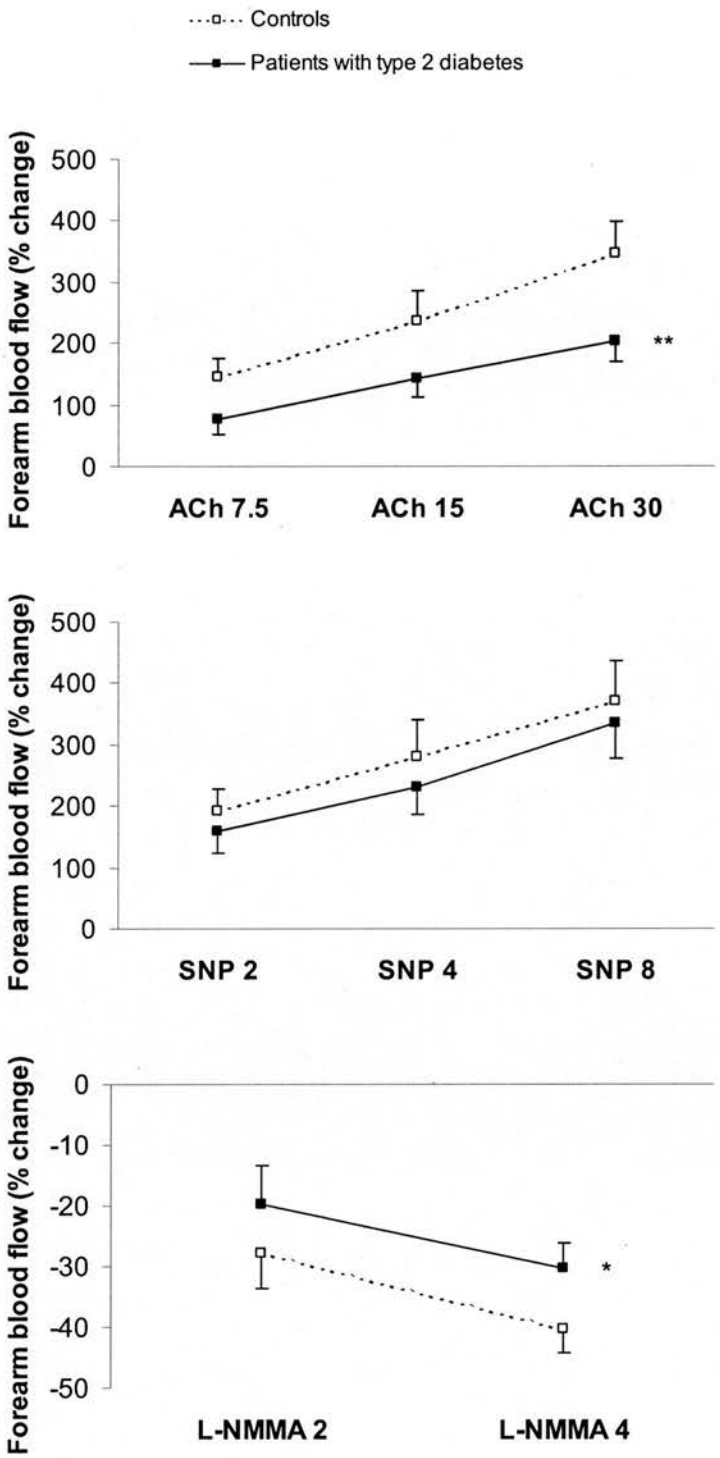
Patients with type 2 diabetes and healthy controls had mean ± SEM augmentation index values of 2.0 ± 1.7 and 13.1 ± 2.3% respectively ($p = 0.006$). Administration of urate oxidase had no acute effects on systemic haemodynamics in either group. Furthermore, augmentation index and haemodynamic variables were similar between pre-dose and 24 h post-dose values, despite lowered serum uric acid concentrations.

Table 40. Mean ± SEM augmentation index and systemic haemodynamic variables before and after administration of urate oxidase 1.5 mg or saline.

		Healthy controls		**Patients with type 2 diabetes**	
		Saline	**Urate oxidase**	**Saline**	**Urate oxidase**
AIx (%)	Pre-dose	2.0 ± 1.5	1.8 ± 1.3	13.4 ± 2.4	12.6 ± 2.3
	24 h	1.9 ± 1.4	2.1 ± 1.1	14.0 ± 2.0	14.5 ± 2.0
Heart rate	Pre-dose	70 ± 3	72 ± 2	76 ± 3	71 ± 2
(min^{-1})	0.5 h	70 ± 3	71 ± 3	75 ± 2	71 ± 2
	1 h	71 ± 3	70 ± 2	76 ± 3	71 ± 2
	24 h	70 ± 3	71 ± 3	71 ± 2	71 ± 2
Systolic BP	Pre-dose	123 ± 4	123 ± 4	124 ± 4	124 ± 5
(mmHg)	0.5 h	124 ± 4	125 ± 4	120 ± 5	128 ± 4
	1 h	122 ± 5	124 ± 4	120 ± 4	125 ± 4
	24 h	123 ± 4	123 ± 4	122 ± 4	121 ± 3
Diastolic BP	Pre-dose	76 ± 2	76 ± 3	76 ± 3	75 ± 2
(mmHg)	0.5 h	75 ± 3	76 ± 3	74 ± 2	75 ± 3
	1 h	76 ± 3	77 ± 3	75 ± 2	80 ± 3
	24 h	77 ± 3	76 ± 3	76 ± 2	76 ± 2

13.3.3 Endothelial Function

Forearm blood flow responses to acetylcholine and L-NMMA were lower in patients with type 2 diabetes than healthy controls ($p = 0.008$ and $p = 0.02$ respectively by ANOVA). Blood flow responses to sodium nitroprusside did not differ significantly between patients with type 2 diabetes and healthy controls. Urate oxidase administration had no effect on forearm blood flow responses in either group.



95% C.I. for control-UA responses AUC is -72 to 252

Figure 25. Mean ± SEM forearm blood flow responses to acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min in patients with type 2 diabetes and healthy controls, 24 h after administration of 10 ml 0.9% saline. $^{*}p < 0.05$, $^{**}p < 0.01$ by ANOVA.

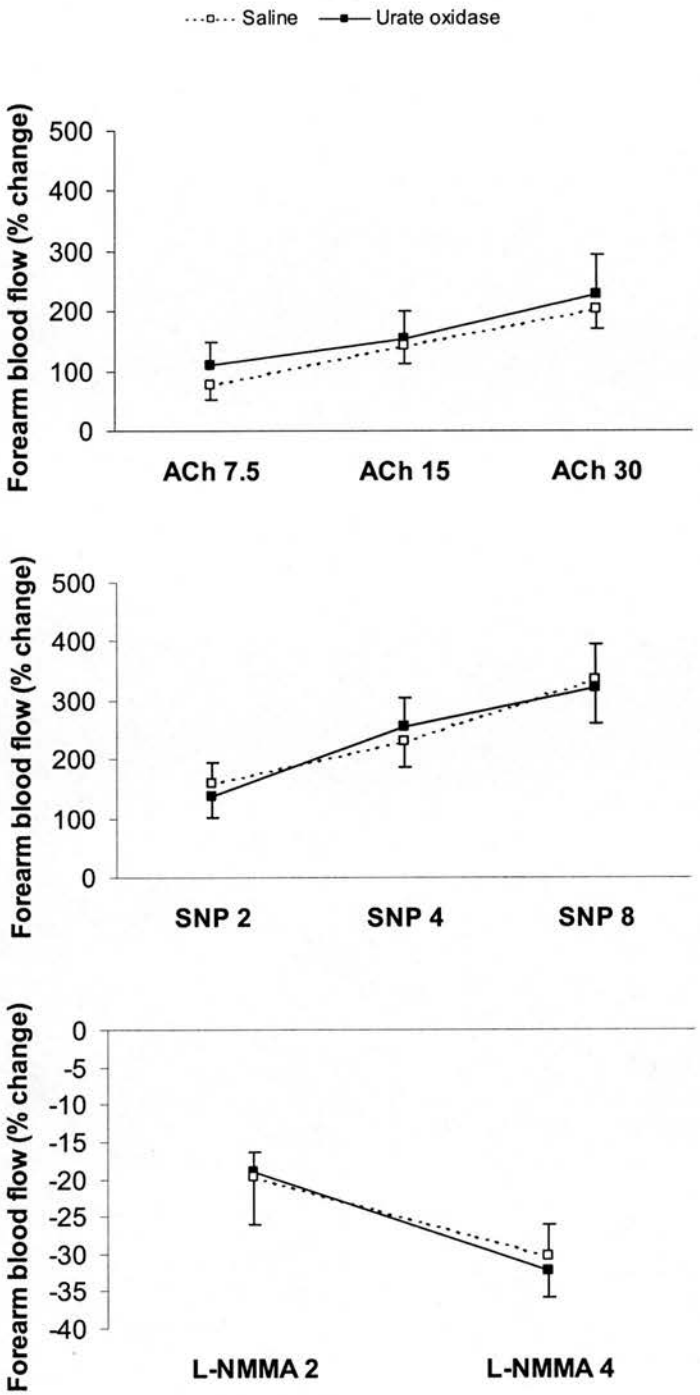


Figure 26. Mean ± SEM forearm blood flow responses to acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min in patients with type 2 diabetes 24 h after administration of urate oxidase 1.5 mg in 10 ml 0.9% saline or saline alone.

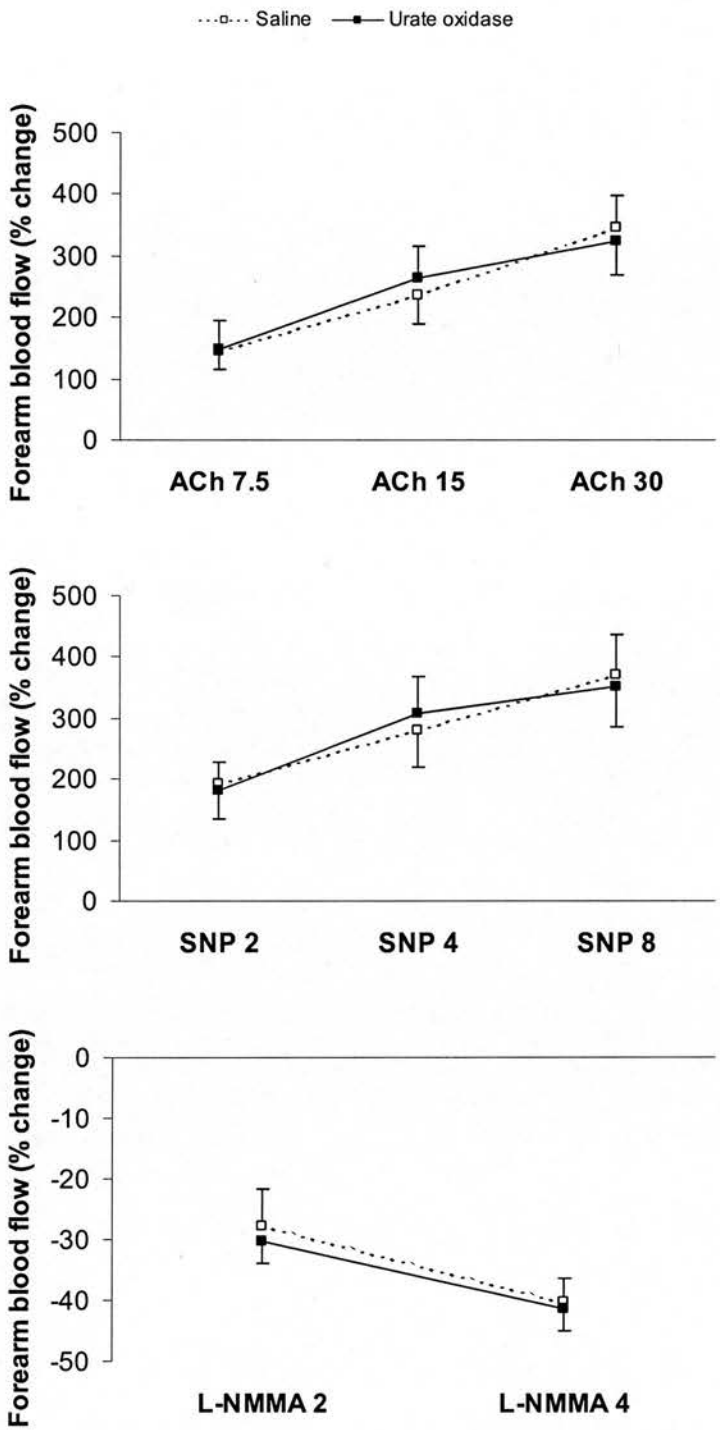


Figure 27. Mean ± SEM forearm blood flow responses to acetylcholine (ACh) 7.5-30 μg/min, sodium nitroprusside (SNP) 2-8 μg/min and L-NMMA 2-4 μmol/min in healthy controls 24 h after administration of urate oxidase 1.5 mg in 10 ml 0.9% saline or saline alone.

13.4 Discussion

The pilot dose-finding study showed that administration of urate oxidase caused a dose-dependent reduction in circulating uric acid concentrations. The maximum dose (1.5 mg) caused around a four-fifths reduction from baseline concentrations at 24 h after single administration. This was followed by a time-dependent restoration of serum uric acid concentrations towards baseline values. On the basis of these findings, the main protocol adopted a single bolus administration of 1.5 mg urate oxidase 24 h before cardiovascular assessment. This dose is lower than that used in the treatment of severe hyperuricaemia, typically 0.2 mg/kg daily for up to 7 days [309]. Furthermore, administration of urate oxidase 24 h before studies of cardiovascular function would coincide with maximal uric acid lowering. Urate oxidase activity is likely to be negligible at this time, based on pharmacokinetic data from animal and human studies [310, 311], thereby avoiding possible confounding effects on vascular function.

Blood glucose control was good in patients with type 2 diabetes, as evidenced by the haemoglobin$_{A1C}$ values at baseline screening, and blood pressure and serum cholesterol concentrations were well matched between patients and controls. Patients with type 2 diabetes had higher serum uric acid concentrations than healthy subjects, in keeping with previously published data. As expected, administration of urate oxidase caused a significant reduction in serum uric acid concentration at 24 h post-dose in patients with diabetes and healthy controls. The effect was of greater magnitude than that reported for other pharmacological interventions in previous studies, including administration of allopurinol.

Baseline augmentation index was significantly higher in patients with type 2 diabetes indicating increased large arterial stiffness in this group, as reported in previous studies. Despite achieving a substantial reduction in circulating uric acid concentrations, urate oxidase administration had no effect on augmentation index values in patients with diabetes or healthy controls. Therefore, lowering serum uric acid concentrations, at least in the acute setting, had no effect on large arterial compliance.

Forearm blood flow responses to acetylcholine were lower in patients with type 2 diabetes, indicating impaired endothelium-dependent nitric oxide-mediated blood flow in this group, as previously described. Responses to L-NMMA were significantly lower in patients with diabetes, indicating that there is also impairment of constitutive endothelium-dependent nitric oxide bioavailability. Forearm blood flow responses to sodium nitroprusside were similar between both groups, although the small subject numbers do not fully exclude a small difference in responsiveness between groups, which might represent a type 2 statistical error. Nonetheless, this observation indicates that endothelium-independent vascular responsiveness to nitric oxide is preserved in patients with type 2 diabetes. These observations are consistent with impaired nitric oxide-mediated endothelial function in patients with type 2 diabetes, as reported in previous studies. Urate oxidase administration had no effect on forearm blood flow responses in either group, demonstrating that lowering serum uric acid concentrations does not improve endothelial function in patients with type 2 diabetes. Previous reports have shown that administration of allopurinol improves endothelial function in patients with type 2 diabetes. The present findings suggest that mechanisms other than uric acid lowering might be responsible for the beneficial effects of allopurinol, for example direct antioxidant effects or inhibition of xanthine oxidase-dependent free radical liberation.

The lack of improvement in endothelial function in the setting of lowered uric acid concentrations is distinct from the rapid restoration of endothelial function caused by amelioration of other major cardiovascular risk factors, for example smoking cessation, blood pressure lowering and cholesterol reduction. The present findings suggest that raised serum uric acid concentrations in patients with type 2 diabetes do not contribute to large arterial stiffness and endothelial dysfunction.

With respect to the control group, neither urate oxidase nor uric acid lowering *per se* appeared to have any influence on vascular function in healthy individuals. This suggests that acute withdrawal of uric acid, and its free radical scavenging antioxidant properties, is not immediately detrimental. It is possible that very small

amounts of uric acid, accompanied by the presence of other antioxidants, are sufficient to maintain vascular integrity in the absence of oxidative stress.

A limitation of the present study is that only short-term measures of large arterial stiffness and endothelial function were examined, and the effects of long-term lowering of serum uric acid concentrations could not be addressed. A further potential limitation is that plasma glucose concentrations were higher in patients with type 2 diabetes than healthy subjects, and hyperglycaemia is a recognized cause of impaired endothelial function. However, plasma glucose concentrations were less than 9.8 mmol/l, and generally well controlled in patients, as evidenced by baseline Hb_{A1C} concentrations. Plasma glucose concentrations were similar between visits and, therefore, unlikely to influence the effect of treatment.

Chapter 14.

Conclusions and Future Research

14.1 Summary

This material presented in this thesis describes the development of an aqueous vehicle to allow administration of uric acid in a research setting. This has allowed the potential cardiovascular effects of raising serum uric acid concentrations to be studied *in vivo*. Acute elevation of local and systemic uric acid concentrations does not exert any direct effect on endothelial function, large arterial stiffness or systemic haemodynamic variables in healthy people. High serum uric acid concentrations confer increased antioxidant capacity, and protect against oxidative stress during acute aerobic exercise in healthy people.

Regular smokers and patients with type 1 diabetes mellitus are characteristically exposed to oxidative stress and vascular dysfunction *in vivo*, thought due to the activity of excess oxygen-derived free radicals. Both groups were found to have lower baseline serum uric acid concentrations than healthy subjects. Raising serum uric acid concentrations, by means of systemic administration, was found to increase serum antioxidant capacity and caused improvement of endothelium-dependent vasodilator forearm responses in regular smokers and patients with type 1 diabetes. These observations suggest that low serum uric acid concentrations might contribute to vascular dysfunction in smokers and patients with type 1 diabetes, as a consequence of diminished antioxidant defences, and that raising serum uric acid concentrations confers protection against oxidative stress.

Patients with type 2 diabetes were found to have higher serum uric acid concentrations than healthy controls, accompanied by impaired endothelium-dependent vascular function and large arterial stiffening. Reducing serum uric acid concentrations, by means of urate oxidase administration, did not influence endothelial function, large arterial stiffness or resting haemodynamic variables in patients with type 2 diabetes mellitus. These observations suggests that high serum uric acid concentrations do not contribute to vascular dysfunction in patients with type 2 diabetes mellitus or healthy subjects.

A major limitation of the studies outlined in the thesis is that only short-term cardiovascular effects of raising or lowering serum uric acid concentrations have been examined. Cardiovascular risk, and the development of atherosclerosis are likely to be influenced by chronic exposure to risk factors. The studies were designed to address the potential effects of high and low serum uric acid concentrations on mechanisms intimately linked to the development of atherosclerosis, which other established cardiovascular risk factors are known to impair. However, this appears to be a valid approach because there is good correlation between short-term and long-term effects of exposure to cardiovascular risk factors and interventions that modify cardiovascular risk [281, 282, 312].

14.2 Clinical Relevance

There is a widely held perception that uric acid is an independent cardiovascular risk factor, based on strong epidemiological associations between high serum concentrations and increased cardiovascular risk. To date, no biologically plausible link between high serum uric acid concentrations and mechanisms of atherosclerosis has been established. If the relationship between high serum uric acid concentrations and cardiovascular risk were causal, then treatment to lower serum uric acid concentrations might provide an effective means of preventing or treating cardiovascular disease.

The studies described in this thesis found that acute exposure to high serum uric acid concentrations caused no detrimental effects on vascular function in healthy people, regular smokers or patients with diabetes. Lowering uric acid concentrations did not cause improved endothelial function in healthy people or those with type 2 diabetes. Moreover, high uric acid concentrations conferred protection against acute oxidative stress, and improved vascular function in patients typically exposed to chronic oxidative stress. These observations show that high serum uric acid concentrations do not exert any acute detrimental effects on endothelial function, an important surrogate marker for atherosclerosis, unlike established major cardiovascular risk factors. These findings do not support the hypothesis that high serum uric acid concentration might be an independent cardiovascular risk factor.

14.3 Future Research

Overall, the data presented in this thesis support the view that high uric acid concentrations are not deleterious to vascular function. Therefore, serum concentrations might serve as a passive marker of risk, and further work is required to explore the potential clinical utility of uric acid measurement, so as to allow better cardiovascular risk stratification.

High uric acid concentrations were found to protect against oxidative vascular damage in the setting of acute oxidative stress, and to restore vascular function in patients exposed to chronic oxidative stress. The mechanisms by which uric acid improved vascular function in regular smokers and patients with type 1 diabetes require further evaluation. These observations suggest that high uric acid concentrations might perhaps be a compensatory response, in the setting of increased cardiovascular risk. Low serum uric acid concentrations might be an important contributory factor to endothelial dysfunction in these groups, and the underlying mechanisms need further exploration.

The increase in serum antioxidant capacity conferred by uric acid administration might have therapeutic potential in conditions associated with oxidative stress. This is likely to have greatest utility if applied in the setting of acute oxidative stress, for example ischaemic stroke or myocardial reperfusion injury, so as to avoid the potential adverse consequences of chronic exposure to high serum uric acid concentrations. Uric acid administration has previously been found to minimise tissue damage associated with acute ischaemia in animal models, and requires confirmation in a clinical setting.

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APPENDIX

Comparison of enhanced chemiluminescence and 'Total Antioxidant Status' assays

A number of assays have been developed for determining the total radical-scavenging antioxidant capacity of biological fluids, which may be preferable to measures of specific antioxidant concentrations or activities. Global antioxidant measurements may be more physiologically relevant because they take account of potential synergistic interactions between antioxidants that appear to be important *in vivo*. Antioxidant capacity determines the ability of serum to oppose the effects of oxidants, including free radicals, and provide a sensitive and reliable marker of defences against oxidative stress *in vivo*, not easily reckonable from measures of individual antioxidant concentrations.

The Total Radical trapping Ability of Plasma (TRAP) assay was one of the earliest methods developed, and is based on inhibition of pro-oxidant activity by antioxidants contained in biological fluids, and has been widely considered the gold standard method [313]. However, it is associated with significant variability and complex methodology, and a variety of more simple, rapid methods based on chemiluminescence or spectrophotometry have been described [314-316]. Assays of antioxidant capacity provide a single measure within a dynamic system, which can be influenced by several metabolic and physiological parameters not related to oxidative stress. Therefore, interpretation depends on the conditions under which the measurement is determined. Measurements of serum antioxidant capacity by chemiluminescence and 'Total Antioxidant Status' assays were compared.

Methods

Baseline antioxidant capacity measurements were compared in 34 samples collected from healthy subjects who had fasted overnight. Additionally, assay measurements were compared across samples collected at 0, 15, 30, 45, 60, 90, and 120 min after commencing systemic administration of uric acid 1000 mg or vitamin C 1000 mg, over 1h, in 10 healthy subjects.

Intra-assay precision was calculated as the mean of duplicate standard deviations ÷ grand mean of the duplicates. The inter-assay coefficient of variation was calculated as the standard deviation of the duplicate means ÷ grand mean of the duplicates. These measures of analytical precision were expressed as percentages and used to represent reproducibility and repeatability respectively for each assay. Antioxidant capacity was compared between assays using Spearman's Rank Correlation.

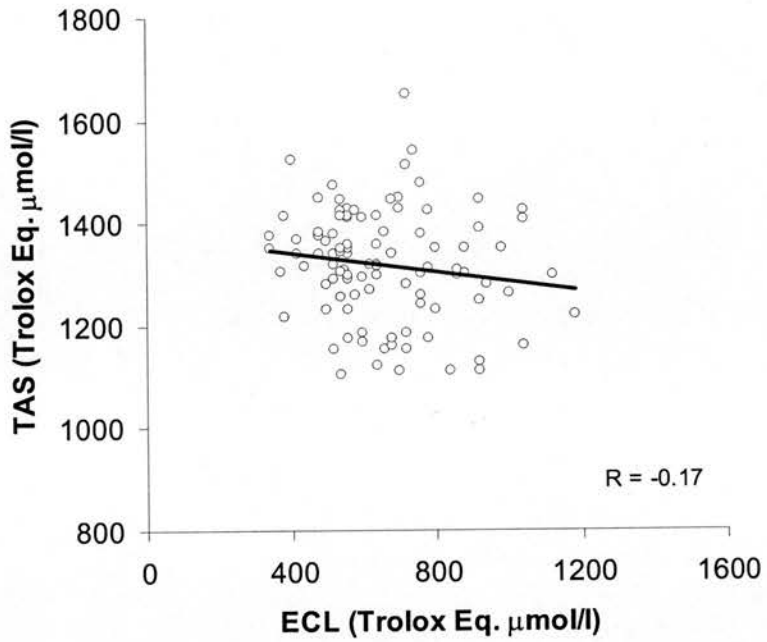
Results

Kolmogorov-Smirnov tests indicated that baseline chemiluminescence and 'Total Antioxidant Status' assay measurements were normally distributed; Kurtosis values were 0.68 and -0.96 respectively, and skewness values were 1.07 and 0.23 respectively. Baseline antioxidant values given by both assays were 469 ± 20 and 1272 ± 13 μmol/l respectively. Intra-assay precision was 9.9 % and 7.9 % respectively, inter-assay precision 8.9% and 5.4% respectively, and inter-individual variation 34.6% and 8.3% respectively for chemiluminescence and 'Total Antioxidant Status' assays.

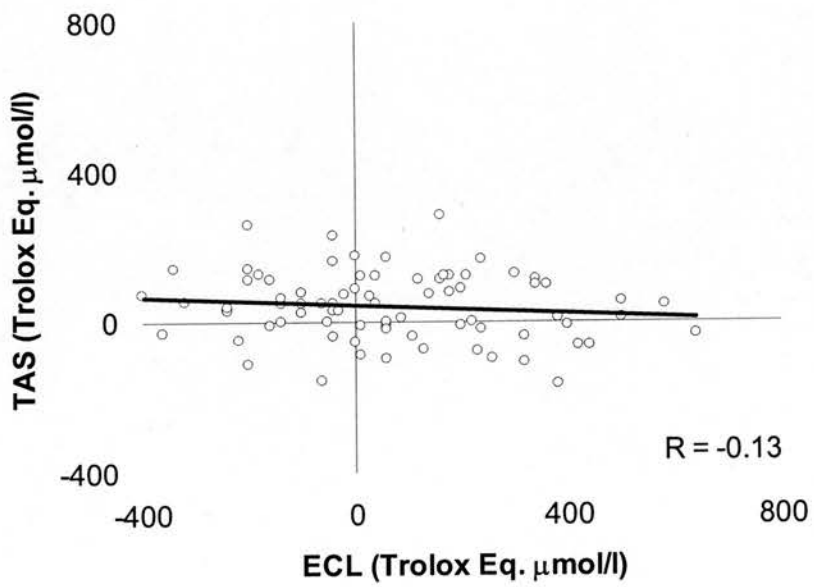
In baseline samples ($n = 34$), correlation between both assays was poor ($R = 0.37$, $p = 0.03$), and correlation between baseline serum uric acid concentrations and antioxidant capacity was poor for chemiluminescence ($R = -0.04$, $p = 0.01$) and 'Total Antioxidant Status' ($R = -0.29$, $p = 0.06$) assays.

During vitamin C administration, correlation between both assays measurements was poor ($R = -0.17$, $p = 0.09$). The increments in antioxidant capacity from baseline also correlated poorly between both assays during vitamin C administration ($R = -0.13$, $p = 0.26$). However, a good correlation was found between measurements given by both assays during uric acid administration ($R = 0.67$, $p < 0.01$), and the increment from baseline values obtained from each assay ($R = 0.79$, $p < 0.01$).

A.

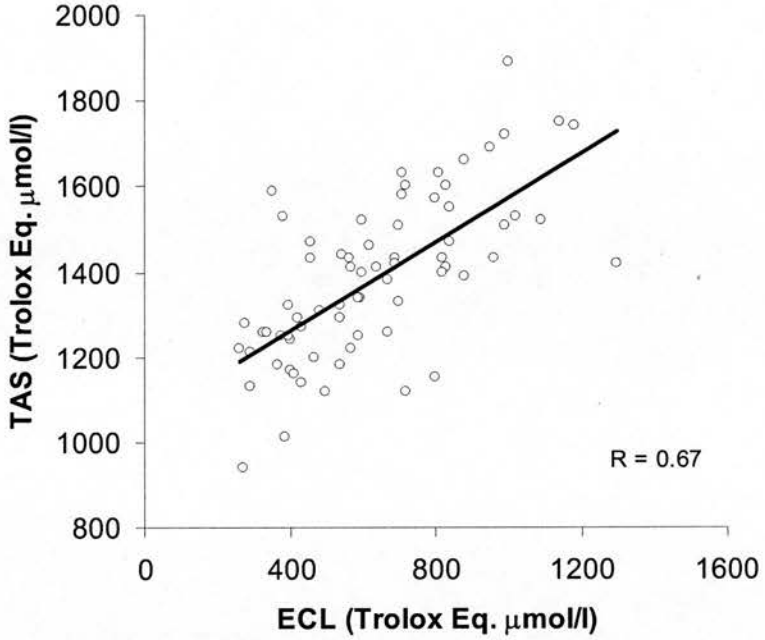


B.



Appendix Figure 1. Correlation between chemiluminescence (ECL) and 'Total Antioxidant Status' (TAS) assays for (A) actual measurements and (B) changes from baseline during systemic administration of vitamin C. $p = 0.088$ ($n = 70$) and $p = 0.261$ ($n = 60$) respectively by Spearman's Rank Correlation.

A.



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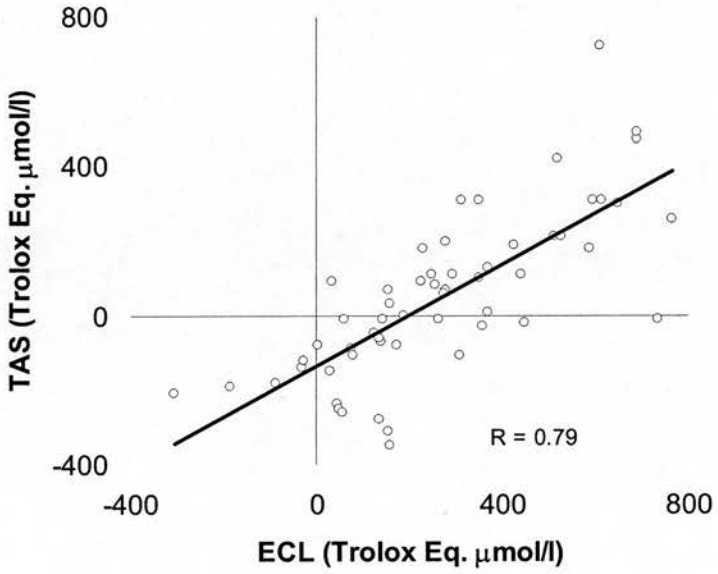

Appendix Figure 2. Correlation between chemiluminescence (ECL) and 'Total Antioxidant Status' (TAS) assays for (A) actual measurements and (B) changes from baseline during systemic administration of uric acid 1000 mg. $p < 0.001$ ($n = 70$) and $p < 0.001$ ($n = 60$) respectively by Spearman's Rank Correlation.

Discussion

There was a poor correlation between the assays, suggesting that they are predominantly influenced by different serum antioxidants. The much greater inter-individual variation in the chemiluminescence assay reflects differences between each method. Chemiluminescence is influenced by antioxidants that are capable of completely suppressing light emission, and is heavily dependent on potent and effective antioxidants, including urate and vitamin C, and is less sensitive to weaker ones. On the other hand, the 'Total Antioxidant Status' assay is influenced by a variety of potent and less potent antioxidants, which exert a cumulative antioxidant effect on indicator absorption. The latter, therefore, provides a global measure of antioxidant capacity that is based on a variety of factors, and does not discriminate between them based on potency. The latter is more likely to be influenced by less potent antioxidant factors, for example albumin, which may account for the lower inter-individual variation seen because albumin concentrations are similar across healthy individuals.

There was close correlation between the assays during uric administration, indicating that both are comparatively sensitive to the contribution of urate to overall serum antioxidant capacity. Surprisingly, no correlation was found between baseline serum urate concentrations and antioxidant capacity measurements given by either assay. Such a relationship may not have been apparent because of the low urate concentrations and narrow range of values found in the present study population. A relationship between urate concentration and antioxidant capacity has previously been demonstrated more clearly in populations with a broad range of serum urate concentrations, for example in patients with sepsis and obesity [316, 317].

Overall there was no correlation between baseline serum antioxidant measurements made by both assays in a healthy population. Both appear sensitive to increases in circulating serum urate concentrations. However, the marked difference in inter-individual variability between the ECL and TAS assay strongly implies that each is sensitive to different antioxidant factors.

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PUBLICATIONS

CARDIOVASCULAR MEDICINE

Hyperuricaemia does not impair cardiovascular function in healthy adults

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Objective: To investigate the possibility that uric acid (UA) can impair endothelial function, an important surrogate for atherosclerosis.

Design: UA was administered locally or systemically to healthy adult men and women in a series of randomised placebo controlled studies. This temporarily raised serum UA concentrations, so that the potential effects of hyperuricaemia on mechanisms of cardiovascular disease could be studied.

Main outcome measures: The effects of UA administration on basal blood flow and responses to locally administered acetylcholine, sodium nitroprusside, and L-N^G-monomethylarginine were studied in the forearm vascular bed with venous occlusion plethysmography. The effects of hyperuricaemia on systemic vascular resistance, large artery compliance, and baroreflex sensitivity were examined by validated non-invasive techniques.

Results: UA administration caused a twofold increase in serum concentrations. However, there were no acute effects on haemodynamic variables, basal forearm blood flow, or nitric oxide dependent endothelial function.

Conclusion: Unlike other risk factors associated with endothelial dysfunction, acute exposure to high concentrations of UA does not impair cardiovascular function in healthy men. These findings do not support a causal link between hyperuricaemia and atherosclerosis.

Uric acid (UA) is formed as a natural product of purine metabolism. Humans are exposed to comparatively high serum and tissue concentrations because of the lack of urate oxidase, an enzyme that is responsible for further metabolism of UA in virtually all other species.[1] A physiological role of UA has been suggested on the basis of its potent antioxidant properties,[2] although this potential benefit may be gained at the cost of an increased risk of joint and kidney disease. Epidemiological studies have identified a strong relation between hyperuricaemia and subsequent cardiovascular disease risk in unselected populations[3–5] and high risk groups, including patients with hypertension, diabetes mellitus, and chronic cardiac failure.[6–8] This relation has been attributed to associations between UA and potential confounding risk factors.[3] However, several epidemiological studies have shown that the predictive power of hyperuricaemia persists, even after considering these risk factors.[4 5] The distinction between UA as a coincidental or causal risk factor is important because, if UA is causal, treatment to lower serum UA concentrations may potentially reduce cardiovascular disease risk. Epidemiological studies are unlikely to resolve this issue. Given the high prevalence of cardiovascular disease, there is a pressing need to identify additional treatable risk factors. The role of UA in the cardiovascular system is poorly understood and potential mechanisms by which it may promote atherogenesis have received little attention.[9]

In health, the endothelium plays a pivotal part in regulating the cardiovascular system through the release of nitric oxide, which causes vasodilatation, inhibits platelet aggregation, and reduces local vascular inflammation.[10] Impaired blood flow responses to endothelium dependent vasodilators are a characteristic finding in patients with any one of several major cardiovascular risk factors and are thought to be an important early step in the development of atherosclerosis.[11 12] Disruption of endothelium dependent nitric oxide bioavailability also manifests as reduced large artery compliance[13] and impaired baroreflex sensitivity (BRS).[14] An inverse relation between serum UA concentration and nitric oxide activity has been identified,[15] and it is therefore possible that UA directly influences endothelial function, either by causing endothelial dysfunction or, as an antioxidant, by protecting against the impairment of endothelial function. One study has reported a lower cardiovascular mortality among patients with heart failure treated with allopurinol and the authors attributed this to lowering of serum UA,[16] although there may be other explanations. The feasibility of administering UA in solution, for clinical studies, has recently been established.[17] The purpose of the present research was to determine whether raised UA concentrations disrupt nitric oxide mediated vascular function, in the same manner as established cardiovascular risk factors. Therefore, the cardiovascular effects of acute hyperuricaemia were studied in healthy volunteers with no identifiable major cardiovascular risk factors.

METHODS

Study group

The local research ethics committee granted approval for the studies and written informed consent was obtained from each participant. The investigation conforms to the principles outlined in the Declaration of Helsinki. Participants were recruited from a community database of healthy volunteers held at the Clinical Research Centre of the University of Edinburgh. Studies were performed in the morning, after an overnight fast, in a quiet room maintained at 24–26°C. Inclusion criteria were male or female sex and age 18 to 45

Abbreviations: ACh, acetylcholine; BP, blood pressure; BRS, baroreflex sensitivity; L-NMMA, L-N^G-monomethylarginine; PI, pulse interval; SNP, sodium nitroprusside; UA, uric acid

years. Exclusion criteria were increased blood pressure (BP) (> 160/100 mm Hg), clinical history of joint, kidney or cardiovascular disease, any medication being taken, regular tobacco use, serum creatinine > 110 μmol/l, or serum UA > 400 μmol/l.

Intra-arterial drug administration

The brachial artery of the non-dominant arm was cannulated with a 27 standard wire gauge steel needle (Cooper's Needle Works Ltd, Birmingham, UK) under local anaesthesia by an aseptic technique. Vasoactive drugs were administered through a 16 gauge epidural catheter (Portex Ltd, Kent, UK) connected to an IVAC P1000 syringe pump (Alaris Medical Ltd, Hampshire, UK).[11] Saline was infused for 30 minutes at the start of the study and for 20 minutes between drugs to establish baseline blood flow. Vasoactive drugs were infused for six minutes at each dose and the rate of infusion was kept constant at 1 ml/min throughout.

Measurement of forearm blood flow

Blood flow was measured in both forearms by venous occlusion plethysmography, as previously described.[11 18] Measurements were taken during the last three minutes of each six minute infusion. The last five recordings were averaged to determine flow in each arm. Responses were expressed as percentage change from baseline, where the ratio of blood flow in the infused to that in the non-infused limbs was considered, to account for any systemic effects.[11]

Systemic vascular resistance

BP was recorded in the dominant arm with a validated oscillometric device (HEM-705CP, Omron, Kyoto, Japan).[19] Cardiac index was assessed by transthoracic bioimpedance (NCCOM3-R7, BoMed, Irvine, California, USA).[20] The systemic vascular resistance index was calculated as the mean arterial pressure divided by the cardiac index.

Pulse wave analysis

The dominant radial artery pulse waveform was assessed by applanation tonometry (SPC-301 micromanometer, Millar Instruments, Houston, Texas, USA). A corresponding aortic pressure waveform was generated by pulse wave analysis software (SphygmoCor, PWV, Sydney, Australia).[21] The augmentation index is a validated measure of large artery stiffness, calculated as the difference between the first and second central systolic BP peaks, expressed as a percentage of pulse pressure.[22]

Spontaneous BRS

Study participants rested supine. Systolic BP was recorded continuously by a Portapres system (TNO, Amsterdam, the Netherlands) and an ECG was measured simultaneously.[23 24] Signals from both devices were recorded over 15 minutes and analysed off line by Chart HRV software (ADInstruments, Hastings, UK). BRS was determined by two independent methods. Parallel increases or decreases in BP and pulse interval (PI) are thought to represent spontaneous baroreflex activity.[25] Sequences of parallel increases or decreases over two or more consecutive beats were analysed and the resulting slope (ΔPI/ΔBP) was used to represent BRS by sequence analysis.[25] Fast Fourier transformation of BP and PI data gave the total spectral power of the variability of each and the formula $(\text{power}_{BP}/\text{power}_{PI})^{1/2}$ gave spontaneous BRS by spectral analysis.[26]

Measurement of biochemical variables

Blood was collected in gel tubes (Sarstedt Ltd, Leicester, UK), allowed to clot, and centrifuged at 1000 *g* for 10 minutes. Serum was separated and UA concentration was determined by a colorimetric dry slide method (Vitros, Ortho-Clinical Diagnostics, Amersham, UK).

Drugs and reagents

UA and lithium carbonate (Ultrapure preparations, Sigma Chemical Company, Poole, UK) were reconstituted in a sterile dextrose solution (Baxter Healthcare, Norfolk, UK) and filtered (0.22 μm Millex, Millipore, Molsheim, France). The drugs used were acetylcholine (ACh; CIBAVision-Ophthalmics, Southampton, UK), sodium nitroprusside (SNP; David Bull Laboratories, Warwick, UK), and L-N^G-monomethylarginine (L-NMMA; Calbiochem-Novobiochem, Nottingham, UK).

Basal forearm blood flow

Six healthy men aged 29 (4) years (SEM) were recruited to a two way randomised placebo controlled study. Participants underwent intra-arterial administration of saline for 20 minutes to establish baseline blood flow, followed by infusion of 0, 0.5, 1.0, 2.0, and 4.0 mg/min UA in 4% dextrose/0.1% lithium carbonate vehicle for six minutes at each dose and for 12 minutes at the maximum dose. Forearm blood flow was assessed at baseline and during each infusion.

Local hyperuricaemia and endothelial function

Ten healthy men were recruited to a two way randomised placebo controlled study. They underwent intra-arterial administration of saline for 30 minutes to establish baseline blood flow, followed by ACh 7.5, 15, and 30 mmol/min, SNP 2, 4, and 8 mmol/min, and L-NMMA 2 and 4 μmol/min, where the order of infusion of ACh and SNP was randomised between subjects. Drug infusions were separated by saline for 20 minutes to allow restoration of basal blood flow. Drugs were infused for six minutes at each dose.[11] UA 2.0 mg/min in 4% dextrose/0.1% lithium carbonate vehicle or vehicle alone was co-infused locally. Effluent venous blood (5 ml) was collected from each forearm during infusion for UA measurement.

Systemic hyperuricaemia and endothelial function

Ten healthy men were recruited to a two way randomised placebo controlled study. An 18 standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa under local anaesthetic. Participants underwent systemic administration of 1000 mg UA in 4% dextrose/0.1% lithium carbonate vehicle or vehicle alone over one hour through the cannula in the non-dominant forearm. Immediately after infusion, endothelial function was studied as described above. Venous blood (5 ml) was drawn from the non-infused forearm cannula at baseline, immediately after infusion, and one hour after infusion for UA measurement.

Systemic haemodynamic variables

Eight healthy men were recruited to a three way randomised placebo controlled study. An 18 standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa under local anaesthetic. Portapres finger cuff and BoMed electrodes were applied. Participants rested supine for 30 minutes, then underwent systemic administration of 1000 mg UA in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, vehicle alone, or 0.9% saline over one hour through the non-dominant forearm cannula. Electrocardiogram and Portapres signals were recorded for BRS determination at baseline and at the end of the infusion. BP, cardiac index, and pulse wave analysis (PWA) were measured at baseline and at 15 minute intervals. Venous blood (5 ml) was drawn through the non-infused forearm cannula at baseline and immediately after infusion for UA measurement.

Data analysis and statistics

Numbers of study participants were determined to give at least 80% power to detect a 10% difference in the primary outcome variables (forearm blood flow response to ACh, augmentation index, and BRS). Responses were compared by two way analysis of variance and paired Student's *t* tests, where appropriate. Significance was accepted at the 5% level in all cases. All values are reported as mean (SEM).

RESULTS

Table 1 shows baseline characteristics of the study participants.

Basal blood flow

Local administration of neither vehicle nor UA had any effect on basal forearm blood flow (fig 1). Local UA and vehicle administrations caused systemic UA concentrations to rise by 62 (13) and −4 (3) μmol/l, respectively ($p < 0.001$); 69 mg (about 410 μmol) UA was administered to each subject and the mean volume of distribution was calculated to be 22.6 (2.0) l.

Local hyperuricaemia and endothelial function

Venous effluent UA concentrations in the infused and non-infused forearms were 384 (7) and 280 (1) μmol/l, respectively, during UA administration ($p < 0.001$) and 290 (4) and 283 (1) μmol/l, respectively, during vehicle administration. This was an increase of 33 (3)% and of −1 (0)% during UA and vehicle administration, respectively ($p < 0.001$). Despite this, responses to ACh, SNP, and L-NMMA were unaltered in the forearm vascular bed (fig 2).

Systemic hyperuricaemia and endothelial function

Serum UA concentrations before, immediately after, and one hour after infusion were 227 (8), 534 (18), and 452 (11) μmol/l, respectively, for UA administration and 224 (27), 220 (27), and 217 (27) μmol/l, respectively, for vehicle administration. These were increases of 145 (19)% and −4 (1)% for UA and vehicle, respectively ($p < 0.001$). Forearm blood flow responses to ACh, SNP, and L-NMMA infusion were not altered by systemic hyperuricaemia (fig 3).

Systemic haemodynamic variables

Baseline versus post-infusion serum UA concentrations were 336 (12) *v* 350 (1) μmol/l, 370 (17) *v* 361 (17) μmol/l, and 370 (16) *v* 627 (23) μmol/l ($p < 0.001$) for saline, vehicle, and UA administration, representing increases from baseline of −4 (1)%, −2 (1)%, and 79 (7)%, respectively ($p < 0.001$). Augmentation index, central systolic BP, BRS by sequence analysis, and BRS by spectral analysis were not altered by systemic hyperuricaemia (table 2). The systemic vascular resistance index increased during all infusions, with a non-significant trend towards lower increases during UA infusion (table 2).

Table 1 Study subjects baseline characteristics

	Local UA and endothelial function	Systemic UA and endothelial function	Systemic UA and haemodynamic variables
Number/men	10/10	10/6	8/8
Age (years)	23 (1)	24 (1)	30 (4)
Systolic BP (mm Hg)	110 (4)	107 (6)	108 (3)
Diastolic BP (mm Hg)	68 (5)	71 (4)	72 (3)
Heart rate (beats/min)	59 (2)	58 (3)	60 (2)
Body mass index (kg/m^2)	23 (1)	22 (1)	23 (0)
Creatinine (μmol/l)	84 (4)	84 (6)	86 (3)
Glucose (mmol/l)	4.6 (0.2)	4.8 (0.1)	4.7 (0.3)
Cholesterol (mmol/l)	4.1 (0.2)	4.1 (0.2)	4.8 (0.2)
Uric acid (μmol/l)	258 (13)	252 (14)	366 (12)

Data are mean (SEM).
BP, blood pressure; UA, uric acid.

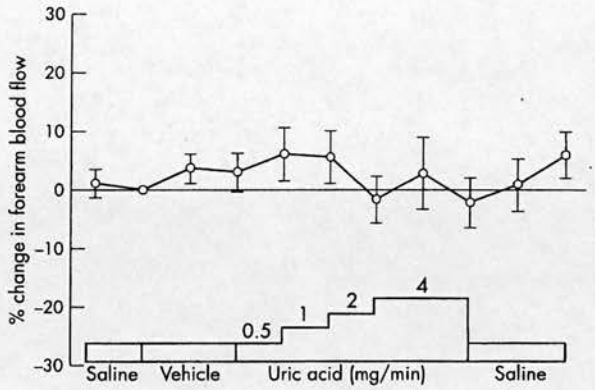

Figure 1 Pilot study. Forearm blood flow, as the ratio of the infused to the non-infused forearm, expressed as percentage change from baseline during local administration of uric acid (UA; 0, 0.5, 1.0, 2.0, and 4.0 mg/min) in the vehicle ($n = 6$).

DISCUSSION

The importance of high serum UA concentration as a marker of increased cardiovascular risk has been recognised for more than 50 years.[27] However, no biologically plausible causal link to atherosclerosis has been shown in vivo. In the current study, UA administration had no effect on basal forearm blood flow or response to L-NMMA, indicating that short lived hyperuricaemia does not have a direct impact on resting vascular tone or basal nitric oxide release. Lack of effect on endothelium dependent and endothelium independent vasodilator responses suggests that high UA concentrations do not affect vascular smooth muscle nitrate responsiveness or stimulate nitric oxide release in health.

There is a strong association between disease states characterised by loss of vascular nitric oxide activity and high serum UA concentrations. The present findings indicate that acute hyperuricaemia does not directly influence constitutive, or stimulated, nitric oxide liberation from the vascular endothelium. A previous study has shown that enhancing vascular nitric oxide bioavailability by L-arginine supplementation causes a reduction in circulating UA concentrations.[15] Therefore, UA may be responsive to vascular nitric oxide activity, consistent with a non-causal association between endothelial dysfunction and increased serum UA concentrations. Furthermore, UA is an important intracellular free radical scavenger during metabolic stress,[28] for example, in vascular smooth muscle and endothelial cells,[29] and circulating concentrations are thought to be responsive to the local redox state.[30 31] Therefore, it is possible that increased UA concentrations are a compensatory response, in view of the antioxidant properties of UA.

Hyperuricaemia coexists with impaired large artery compliance in several disease states characterised by reduced vascular nitric oxide bioavailability. UA had no impact on the large conduit vessels that determine pulse waveform conduction, suggesting that high concentrations are not causally linked to increased vascular stiffness. BRS, determined by two discrete methods,[25 26] was unaffected by UA, consistent with the lack of effect on constitutive nitric oxide activity and large artery compliance, which are important vessel wall properties that can influence BRS. The lack of effect on BRS also indicates that baroreceptor function, and indeed cardiac sensitivity to autonomic outflow, was not affected by local

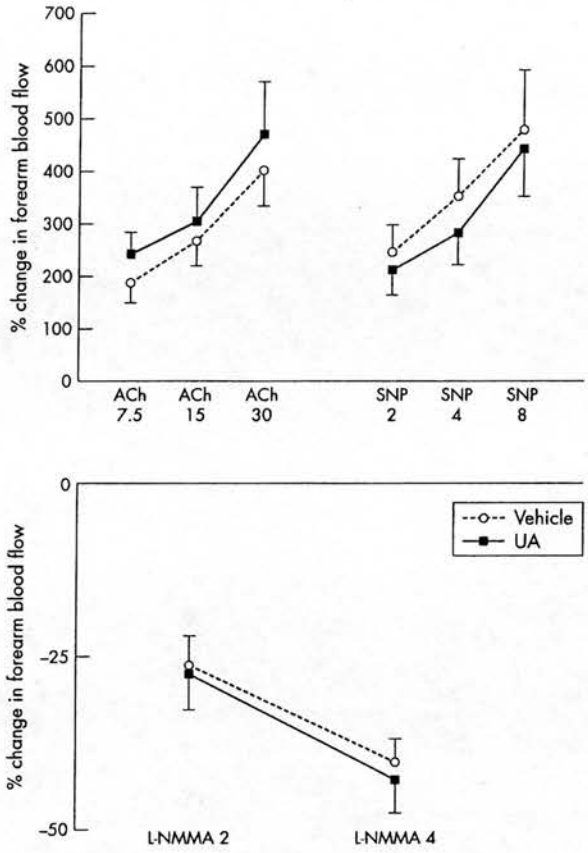

Figure 2 Forearm blood flow responses to acetylcholine (ACh) 7.5, 15, and 30 μg/min, nitroprusside (SNP) 2, 4, and 8 μg/min, and L-N^G-monomethylarginine (L-NMMA) 2 and 4 μmol/min, as a ratio of the infused to the non-infused forearm and expressed as percentage change from baseline during local co-administration of 2 mg/min UA in vehicle or of vehicle alone (p = 0.74, 0.47, and 0.87, respectively; n = 10).

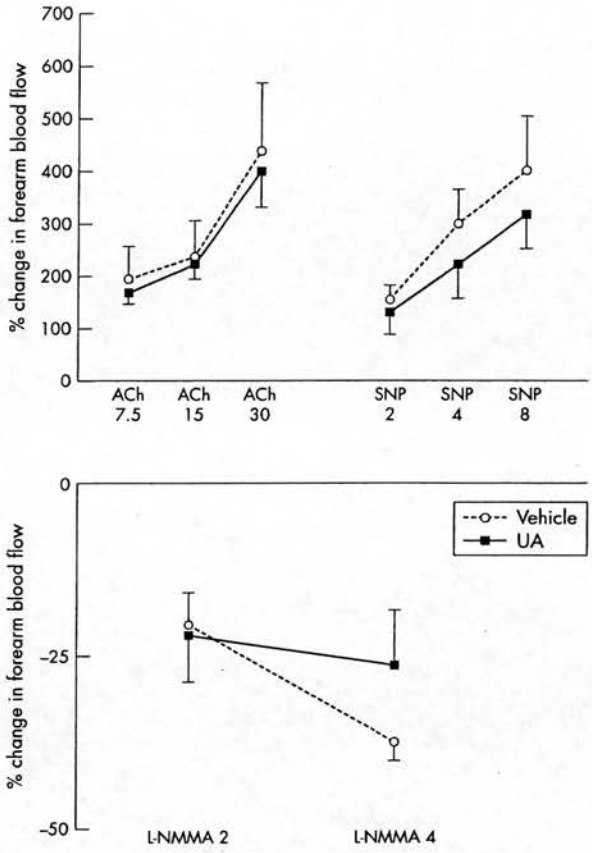

Figure 3 Forearm blood flow responses to ACh 7.5, 15, and 30 μg/min, SNP 2, 4, and 8 μg/min, and L-NMMA 2 and 4 μmol/min, as a ratio of the infused to the non-infused forearm and expressed as percentage change from baseline after systemic administration of 1000 mg UA in vehicle or of vehicle alone (p = 0.87, 0.65, and 0.38, respectively; n = 10).

increases in UA concentration, irrespective of vessel wall conditions.

There are several important limitations of these findings. This series of acute, mechanistic studies did not address the effects of chronic exposure to increased serum UA concentrations, which may be a more important determinant of future cardiovascular risk. However, it is less feasible to examine chronic hyperuricaemia in a controlled manner because of the potential risks of joint and kidney disease. A further potential limitation is that the current studies addressed the effects of an acute increase of UA in a young, healthy population, free from major cardiovascular risk factors. Nonetheless, if UA were an independent causal risk factor for atherosclerosis, then its presence should be expected to impair endothelial function. Other atherosclerotic risk factors cause acute impairment of endothelial function in young, healthy people—for example, increased homocysteine concentrations after methionine administration[32] or ingestion

Table 2 Systemic haemodynamic variables before and after systemic administration of 1000 mg UA in vehicle, vehicle alone, or saline (n = 8)

	Saline		Vehicle		UA	
	Baseline	Postinfusion	Baseline	Postinfusion	Baseline	Postinfusion
SBP (mm Hg)	96 (3)	97 (3)	93 (2)	97 (5)	96 (4)	100 (4)
DBP (mm Hg)	61 (2)	65 (2)	63 (3)	68 (3)	62 (4)	71 (3)
HR (beats/min)	55 (3)	53 (3)	57 (4)	58 (3)	58 (3)	59 (2)
AIx (%)	−6.7 (6.0)	−3.1 (6.1)	−2.6 (6.0)	−1.1 (6.1)	−3.6 (5.1)	−6.1 (6.2)
CI (l/min/m^2)	3.3 (0.2)	3.0 (0.2)	3.3 (0.4)	3.3 (0.3)	3.1 (0.2)	3.2 (0.3)
SVRI (au)	12.0 (1.0)	13.7 (0.9)	10.2 (1.5)	11.7 (2.0)	12.3 (1.0)	13.0 (1.0)
BRS_{seq} (ms/mm Hg)	22.3 (4.7)	21.2 (4.0)	19.7 (4.9)	22.8 (5.0)	21.8 (4.4)	20.3 (4.4)
BRS_{spec} (ms/mm Hg)	24.3 (5.8)	19.4 (3.4)	22.2 (6.6)	26.8 (6.4)	23.4 (5.0)	21.7 (5.9)

Data are mean (SEM).
AIx, augmentation index; au, arbitrary units; BRS_{seq}, baroreflex sensitivity by sequence analysis; BRS_{spec}, baroreflex sensitivity by spectral analysis; CI, cardiac index; DBP, diastolic blood pressure; HR, heart rate; SBP, systolic blood pressure; SVRI, systemic vascular resistance index

of a fatty meal.[33] Additionally, amelioration of established risk factors allows rapid restoration of endothelial function, including correction of hypertension, hypercholesterolaemia, or hyperhomocysteinaemia.

A further limitation is that the effects of raising UA concentrations on endothelial function were studied only in healthy men. Previous studies have shown that the relation between hyperuricaemia and increased cardiovascular risk is more apparent in women, in both unselected populations[34] and those with established coronary artery disease.[35] Additional research is required to investigate the effects of raised serum UA concentrations on endothelial function in women. Furthermore, the effects of raised serum UA concentrations merit further investigation in men and women with established cardiovascular risk factors.

The effects of raising UA concentrations may be modest because of the comparatively high background concentrations to which humans, as a species, are ordinarily exposed. Further work is required to establish the cardiovascular effects of lowering serum UA concentrations. Xanthine oxidase inhibitors—for example, allopurinol—cause a modest reduction in circulating UA concentrations. Xanthine oxidase activity produces hydrogen peroxide, an important source of free radicals in vivo,[36] which confounds the relation between xanthine oxidase inhibitors and UA lowering. Methods that lower UA directly should allow more useful interpretation of any cardiovascular effects—for example, using urate oxidase, which causes rapid and substantial reductions in circulating UA concentrations.[37]

In summary, high serum UA concentrations, at least in the acute setting, do not impair cardiovascular function in healthy men. These findings do not support a causal role for UA in the development of atherosclerosis.

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Comparison of spectrophotometric and enhanced chemiluminescent assays of serum antioxidant capacity

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Abstract

Background: Over recent years, interest in total antioxidant capacity measurement in biological fluids has increased. A number of assays are now available, and we wished to compare an enhanced chemiluminescence (ECL) method to a spectrophotometric method, the total antioxidant status (TAS) assay. *Methods*: Serum urate concentration, ECL and TAS were measured in 34 healthy subjects. Additionally, 10 subjects participated in a two-way, randomised crossover study, and received urate 1000 mg or vitamin C 1000 mg intravenously over 1 h. Serum ECL and TAS were measured at 0, 15, 30, 45, 60, 90 and 120 min after commencing infusion. *Results*: Baseline measurements were poorly correlated between ECL and TAS assays, and between serum urate concentration and each antioxidant assay. There was good correlation between the change in antioxidant capacity detected by both assays during urate infusion ($R=0.79$, $p<0.001$, $n=60$), but not vitamin C infusion. *Conclusions*: ECL and TAS measures of serum antioxidant capacity correlate poorly in a healthy population, although both are sensitive to increases in circulating urate concentrations. Therefore, ECL and TAS appear sensitive to different factors. The comparative strengths and weaknesses of various antioxidant assays should be reviewed.

Keywords: Total antioxidant status; Uric acid; Ascorbic acid; Randomised controlled trial

1. Introduction

Recently, there has been increasing interest in the contribution of free radical-mediated oxidative stress to several disorders, including atherosclerosis, diabetes mellitus, and degenerative neurological diseases [1–3]. This has stimulated interest in the role of protective physiological antioxidants, and assay methods used for their quantification. Antioxidants act in various ways to prevent oxidative damage; however, it is scavenging or 'chain-breaking' antioxidants that are believed to confer greatest protection, and these include a variety of compounds such as ascorbate, alpha-tocopherol, urate, thiols and flavinoids [4,5]. A number of assays have been developed for determining the total radical-scavenging antioxidant capacity of biological fluids, which may be preferable to measures of specific antioxidant concentrations or activities. Global antioxidant measurements may be more physiologically relevant because they take account of potential synergistic interactions between

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antioxidants that appear to be important in vivo [6]. Antioxidant capacity determines the ability of serum to oppose the effects of oxidants, including free radicals. These assays are thought to provide a sensitive and reliable marker of defences against oxidative stress in vivo, not easily reckonable from measures of individual antioxidant concentrations.

The total radical trapping ability of plasma (TRAP) assay was one of the earliest methods developed, and is based on inhibition of pro-oxidant activity by antioxidants contained in biological fluids, and has been widely considered the gold standard method [7]. However, it is associated with significant variability and complex methodology, and a variety of more simple, rapid methods based on chemiluminescence or spectrophotometry have been described [6,8,9]. Assays of antioxidant capacity provide a single measure within a dynamic system, which can be influenced by several metabolic and physiological parameters not related to oxidative stress. Therefore, interpretation depends on the conditions under which the measurement is determined. We wished to compare two separate assays used to determine serum antioxidant capacity, and to compare the performance of each assay during systemic administration of one of two important aqueous antioxidants, urate or vitamin C.

2. Methods

2.1. Study subjects

Subjects were recruited from a community volunteer database held at the Clinical Research Centre of the University of Edinburgh. Inclusion criteria were men and women aged 20–40 years. Exclusion criteria were regular smoking, blood pressure>140/90 mm Hg, cholesterol>6.5 mmol/l, diabetes mellitus, serum urate>250 μmol/l, creatinine>100 μmol/l, taking any regular prescription medication or over-the-counter medication in the week before the study, or pregnancy determined by a human chorionic gonadotrophin-based urinary test. The study was in performed in accordance with the principles of the Declaration of Helsinki. The study protocol had been approved by the local research ethics committee, and all subjects provided written informed consent before participation.

2.2. Study protocol

Thirty-four healthy subjects fasted overnight and studies were performed in the morning in a quiet, comfortable, temperature-controlled room (24–26 °C). After resting supine for 20 min, a 5 ml blood sample was collected from an antecubital fossa vein into a serum gel tube (Sarstedt, Leicester, UK) using aseptic technique. Samples were allowed to clot, then centrifuged at $1000 \times g$ for 10 min at 4 °C. Serum was decanted immediately, and stored at −40 °C prior to determination of antioxidant capacity. All samples were labelled with an anonymous identifier so that the investigator performing the assays was blinded to the subject, sample time, and treatment.

Ten subjects participated in a randomised, double-blind, two-way crossover study. Each underwent insertion of an 18-standard gauge venous cannula into a large vein in both antecubital fossae using local anaesthesia and aseptic technique. Either 1000 mg vitamin C (5682 μmol) or 1000 mg uric acid (5952 μmol) in 500 ml 4% dextrose/0.1% lithium carbonate vehicle was infused via the nondominant forearm cannula over 60 min. Lithium carbonate based vehicle allows stable dissolution of urate in aqueous solution [10], and does not exert a direct effect on serum antioxidant capacity measured by either the ECL or TAS method [11]. Venous blood samples (5 ml) were drawn via the other forearm cannula at 0, 15, 30, 45, 60, 90 and 120 min after the start of the infusion.

2.3. Urate measurement

Serum urate concentrations were determined using an automated colorimetric dry slide assay (Vitros Chemistry Products, Johnson & Johnson, USA).

2.4. Chemiluminescence-based antioxidant assay

Enhanced chemiluminescence (ECL) is based on the emission of light that occurs when luminol is oxidised by hydrogen peroxide, catalysed by horseradish peroxidase, and enhanced by phenol [9]. Light emission is dependent on continuous production of free radicals, and is extinguished by biological fluids for a lag period that is proportional to their antioxidant content. A luminometer (Model 1251; BioOrbit, Turku, Finland) was used to establish the duration

Table 1
Baseline characteristics of the study population

Characteristic	Mean ± S.E.M.
Number (male)	34 (20)
Age (years)	26 ± 1
Height (m)	169 ± 3
Weight (kg)	63 ± 3
Systolic blood pressure (mm Hg)	106 ± 5
Diastolic blood pressure (mm Hg)	69 ± 3
Serum urate (μmol/l)	259 ± 3
Serum cholesterol (mmol/l)	4.2 ± 0.2
Serum creatinine (μmol/l)	87 ± 5

of the lag period caused by addition of serum. This was calibrated against a standard curve generated by addition of 0.16, 0.32, 0.48 and 0.64 μmol/l Trolox, a water-soluble tocopherol analogue (6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid, Sigma, Dorset, UK), and serum antioxidant capacity was expressed as μmol/l of Trolox equivalent.

2.5. Spectrophotometry-based antioxidant assay

The total antioxidant status (TAS) assay (Randox Laboratories, Crumlin, UK) is based on the activity of the free radical ferrylmyoglobin, which is formed by oxidation of metmyoglobin by hydrogen peroxide [12,13]. Ferrylmyoglobin interacts with the chromogen ABTS (2,2′-amino-di-[3-ethylbenzthiazole sulfonate]) to form a blue-green chromophore radical (ABTS.), which has maximal absorbance at 417, 645, 734 and 815 nm. Addition of serum causes a reduction in ABTS. radical activity that is proportional to its antioxidant content. ABTS. absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, East Sussex, UK), and measurements were calibrated against a Trolox standard curve. Serum antioxidant capacity was expressed as μmol/l Trolox equivalent.

2.6. Data analysis

Intra-assay precision was calculated as the mean of duplicate standard deviations/grand mean of the duplicates. The inter-assay coefficient of variation was calculated as the standard deviation of the duplicate mean/grand mean of the duplicates. These measures of analytical precision were expressed as percentages and used to represent reproducibility and repeatability, respectively, for each assay. Antioxidant capacity determined by ECL and TAS assays was compared using Spearman's rank correlation. Statistical significance was accepted at the 5% level in all cases.

3. Results

Baseline characteristics of the study subjects are shown in Table 1. Kolmogorov–Smirnov tests indicated that baseline ECL and TAS antioxidant measurements were normally distributed. Baseline antioxidant

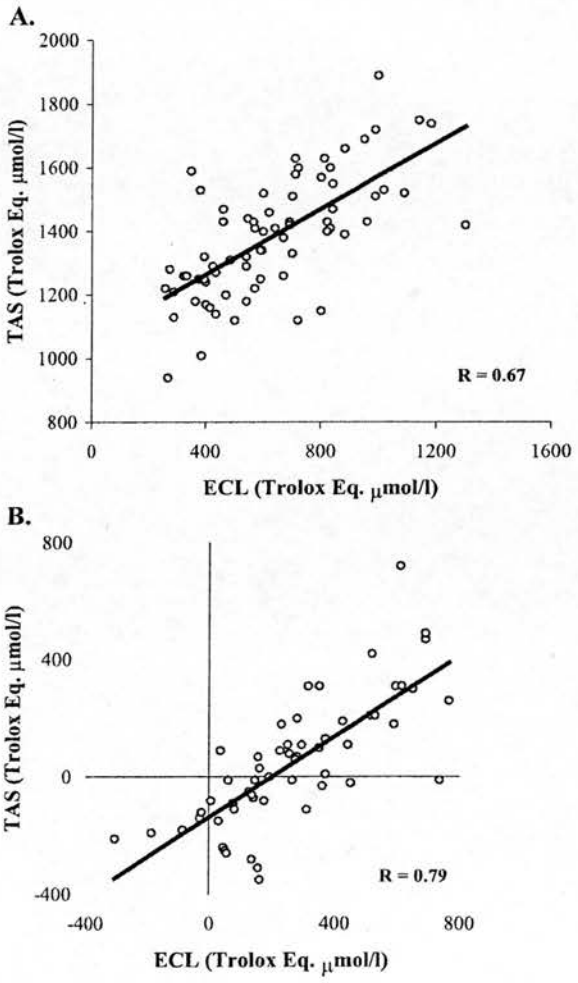

Fig. 1. Correlation between ECL and TAS assay methods (as μM Trolox equivalent) for (A) actual measurements and (B) changes from baseline during systemic administration of urate 1000 mg. $p < 0.001$ ($n = 70$) and $p < 0.001$ ($n = 60$), respectively, by Spearman's rank correlation.

values given by the ECL and TAS assays for the study population were 469 μmol/l (95% confidence interval 428–511 μmol/l) and 1272 μmol/l (1244–1299 μmol/l), respectively. Precision values were calculated across a wide range of antioxidant measures for both ECL (260–1136 μmol/l) and TAS (940–1890 μmol/l) assays. Values for intra-assay precision were 9.9% and 7.9%, respectively, inter-assay precision 8.9% and 5.4%, and inter-individual variation 34.6% and 8.3% for ECL and TAS assays, respectively.

In samples collected from the whole study population ($n=34$), correlation between the ECL and TAS assays was poor ($R=0.37$, $p=0.03$), and correlation between baseline serum urate concentrations and antioxidant capacity was poor for ECL ($R=-0.04$, $p=0.01$) and TAS ($R=-0.29$, $p=0.06$) assays.

During vitamin C administration, correlation between both assays measurements was poor ($R=-0.17$, $p=0.09$). The increments in antioxidant capacity from baseline also correlated poorly between both assays during vitamin C administration ($R=-0.13$, $p=0.26$). However, a good correlation was found between measurements given by the ECL and TAS assays during urate administration ($R=0.67$, $p<0.01$), and the increment from baseline values obtained from each assay ($R=0.79$, $p<0.01$) (Fig. 1).

4. Discussion

The baseline characteristics indicate a young, healthy population with normal blood pressure, body mass index, and serum glucose and cholesterol concentrations. The comparatively high baseline serum antioxidant capacity measurements are consistent with a healthy population, free of any major cardiovascular risk factors that can influence serum antioxidant capacity [12,13]. There was a poor correlation between the ECL and TAS assays, suggesting that they are predominantly influenced by different serum antioxidants. The much greater CV in the ECL assay compared to the TAS assay is consistent with previous reports [13], and provides further evidence that each is influenced by different factors. The discrepancy is likely to arise because of the differing methodology underlying each assay. The ECL assay is influenced by antioxidants that are capable of completely suppressing light emission. Therefore, it is heavily dependent on the presence of potent and effective antioxidants, including urate and vitamin C [1], and less sensitive to weaker ones. On the other hand, the TAS assay is influenced by a variety of potent and less potent antioxidants, which exert a cumulative antioxidant effect on indicator absorption. The latter, therefore, provides a global measure of antioxidant capacity that is based on a variety of factors, and does not discriminate between them based on potency. Therefore, the TAS assay, as with other spectrophotometric assays, is more easily influenced by less potent antioxidant factors including albumin, which may account for as much as 43% of the total measurement. There is little variation in serum protein concentration within a healthy population and the effects of this are seen clearly in our results, where inter-individual variation of reported antioxidant capacity was considerably lower using the TAS (8.3%) rather than ECL (34.6%) method. Relative insensitivity of the ECL assay to protein thiols could be interpreted at first sight as a potential disadvantage. However, the physiological relevance of protein thiols is still a matter of some contention, and their potential role has been less clearly defined than other more potent antioxidants, for example, vitamins C and E [1]. The ECL assay may offer important advantages conferred by its ability to measure the activity of highly efficient antioxidant molecules, without being flooded by high concentrations of relatively inefficient ones.

There was close correlation between ECL and TAS assays during urate administration when considering actual antioxidant measurements, and changes in antioxidant capacity from baseline measurements. These findings indicate that both assays are comparatively sensitive to the contribution of urate to overall serum antioxidant capacity, which is in line with previously reported data. Surprisingly, no correlation was found between baseline serum urate concentrations and antioxidant capacity measurements given by either assay. Such a relationship may not have been apparent because of the low urate concentrations and narrow range of values found in the present study population. A relationship between urate concentration and antioxidant capacity has previously been demonstrated more clearly in populations with a broad range of serum urate concentrations, for example, patients with sepsis or obesity [14,15].

We conclude that there is no correlation between the serum antioxidant measurements made by the ECL and TAS assays in a healthy population. Both assays appear to be sensitive to increases in circulating serum urate concentrations. However, the marked difference in inter-individual variability between the ECL and TAS assay strongly implies that each is sensitive to different antioxidant factors. We believe that it is important to avoid confusion in the literature regarding this interesting field of research. Therefore, we suggest that there should be a systematic comparative review of these and other global antioxidant assays, so that their relative strengths and weaknesses can be identified.

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Uric acid reduces exercise-induced oxidative stress in healthy adults

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ABSTRACT

Uric acid (UA) possesses free-radical-scavenging properties, and systemic administration is known to increase serum antioxidant capacity. However, it is not known whether this protects against oxidative stress. The effects of raising UA concentration were studied during acute aerobic physical exercise in healthy subjects, as a model of oxidative stress characterized by increased circulating 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$) concentrations. Twenty healthy subjects were recruited to a randomized double-blind placebo-controlled crossover study, and underwent systemic administration of 0.5 g of UA in 250 ml of 0.1 % lithium carbonate/4 % dextrose vehicle or vehicle alone as control. Subjects performed high-intensity aerobic exercise for 20 min to induce oxidative stress. Plasma 8-iso-$PGF_{2\alpha}$ concentrations were determined at baseline, after exercise and after recovery for 20 min. A single bout of high-intensity exercise caused a significant increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations from 35.0 ± 4.7 pg/ml to 45.6 ± 6.7 pg/ml ($P < 0.01$). UA administration raised serum urate concentration from 293 ± 16 to 487 ± 16 μmol/l ($P < 0.001$), accompanied by increased serum antioxidant capacity from 1786 ± 39 to 1899 ± 45 μmol/l ($P < 0.01$). UA administration abolished the exercise-induced elevation of plasma 8-iso-$PGF_{2\alpha}$ concentrations. High UA concentrations are associated with increased serum antioxidant capacity and reduced oxidative stress during acute physical exercise in healthy subjects. These findings indicate that the antioxidant properties of UA are of biological importance *in vivo*.

INTRODUCTION

Oxidative stress is characterized by excess free radical activity and is believed to play an important role in the development of atherosclerosis [1]. This has stimulated interest in the possibility that antioxidants could offer protective effects within the cardiovascular system. Until recently, the lack of an adequate biological marker of oxidative stress *in vivo* has limited studies of the efficacy of antioxidant supplementation. A number of oxidative reaction products have been explored, including lipid hydroperoxides, malonyl dialdehyde and prostaglandin F_2-like substances, so called F_2-isoprostanes. F_2-isoprostanes are a family of compounds formed by non-enzymic oxidative modification of arachadonic acid, and result from free radical attack of phospholipids in cell membranes or circulating low-density lipoproteins [2]. They are formed *in situ* in the cell membrane, from which they are cleaved by phospholipase, and circulate in a free form or as a phospholipid-bound ester in a bound/free ratio of approx. 1:2 [3]. Of the F_2-isoprostanes, 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$; also known as $iPF_{2\alpha}$-III) has been shown to be capable of exerting a number of biological effects, such as smooth muscle contraction, vasoconstriction and increased platelet aggregability [4,5]. However, the relevance of these effects remains

Key words: antioxidant, ergometric exercise, isoprostane, oxidative stress, systemic vascular resistance, uric acid.

Abbreviations: ABTS, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid); BP, blood pressure; CI, cardiac index; 8-iso-$PGF_{2\alpha}$, 8-iso-prostaglandin $F_{2\alpha}$; UA, uric acid.

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uncertain, because they are caused by significantly higher 8-iso-$PGF_{2\alpha}$ concentrations than typically found in human plasma. Concentrations of 8-iso-$PGF_{2\alpha}$ are stable in isolated body fluids and quantification in plasma or urine provides a marker of free radical generation *in vivo*. Its concentrations in biological fluids are increased in the presence of major cardiovascular risk factors characterized by oxidative stress, for example hypercholesterolaemia [6], cigarette smoking [7] and diabetes mellitus [8]. Elevated 8-iso-$PGF_{2\alpha}$ concentrations in these conditions support the hypothesis that oxidative modification is an important early step in the development of atherosclerosis. Circulating 8-iso-$PGF_{2\alpha}$ concentrations provide a non-invasive quantitative measure of lipid peroxidation and represent a major advance in assessment of vascular oxidative stress *in vivo*.

Moderately intense physical exercise increases oxygen utilization and causes excess oxygen-derived free radical liberation through mitochondrial lipid peroxidation, neutrophil degranulation and up-regulation of xanthine oxidase activity, which liberates superoxide ($O_2^{\bullet}$) [9]. Intense physical exercise provides a model for studying the effects of acute oxidative stress *in vivo* and has been shown to increase susceptibility of low-density lipoprotein to oxidation and impair endothelial-dependent vasodilatation in the forearm vascular bed [10,11]. Oxidative stress during acute exercise is characterized by elevated circulating 8-iso-$PGF_{2\alpha}$ concentrations, which has provided an opportunity to examine the effects of antioxidant administration on free radical activity *in vivo* [9]. Supplementation with vitamin C or E increases resistance to exercise-induced lipid peroxidation in healthy individuals [12]. However, β-carotene does not reduce oxidative stress during acute physical exercise [13], and combined administration of vitamin C and *N*-acetyl-cysteine paradoxically increase 8-iso-$PGF_{2\alpha}$ concentrations after acute eccentric exercise, despite increased serum antioxidant capacity [14]. Glutathione and ubiquinol supplementation reduce oxidative stress in animal models of acute exercise [15], but their effects have not been fully characterized in humans.

Uric acid (UA) is an abundant aqueous antioxidant that accounts for almost two thirds of all free-radical-scavenging activity in human serum [16]. UA reacts with oxygen-derived free radicals and becomes oxidized in skeletal muscle during high-intensity exercise [17]. Intracellular UA concentrations are rapidly replenished by uptake from plasma after exercise [18]. A previous study [19] found a significant inverse relationship between serum UA concentrations and oxidative stress during acute aerobic exercise. These observations suggest that high UA concentrations could confer protection against free radical activity *in vivo* and indicate that UA may be of biological importance in the setting of acute oxidative stress. The feasibility of systemic UA administration, to increase serum antioxidant capacity, has recently been established in a research setting [20]. The present study aimed to characterize the effects of elevated serum UA concentrations on oxidative stress induced by acute physical exercise, reflected by plasma 8-iso-$PGF_{2\alpha}$ concentrations.

METHODS

Subjects

Twenty healthy men and women were recruited from a community database of healthy subjects held at the Clinical Research Centre of the University of Edinburgh. The protocol was reviewed and approved by the Local Research Ethics Committee, and written informed consent was obtained from each participant. Inclusion criteria were men or women aged 18–45 years. Exclusion criteria were elevated blood pressure (BP; > 160/100 mmHg), clinical history of joint, kidney or cardiovascular disease, those taking any regular medication or non-prescription medication in the past week, serum creatinine > 110 mmol/l, or serum UA > 400 mmol/l.

Drugs and reagents

UA and lithium carbonate (Ultrapure preparations; Sigma, Poole, Dorset, U.K.) were reconstituted in sterile dextrose solution (Baxter Healthcare, Thetford, Norfolk, U.K.), filtered (0.22 mm Millex filter; Millipore, Molsheim, France), and prepared on the day of administration. UA (0.5 g) was dissolved in 250 ml of 0.1 % lithium carbonate/4 % dextrose vehicle. This vehicle was chosen because it allows stable dissolution of UA [21] and does not influence serum antioxidant capacity [20].

Protocol

Subjects were enrolled in a randomized two-way double-blind placebo-controlled crossover study. Investigations were performed in a quiet room maintained at 24–26 °C. An 18-standard gauge venous cannula was inserted into a suitable vein in each antecubital fossa, under local anaesthetic using aseptic technique. Subjects remained seated for 20 min to establish baseline haemodynamic conditions, and underwent systemic administration of 0.5 g of UA in 250 ml of vehicle or 250 ml of vehicle alone over 20 min via the non-dominant forearm cannula. Subjects performed lower limb exercise using an upright electronically braked ergometric cycle machine (Ergometry System 380B; Siemens-Elema, Solna, Sweden). Pedalling rate was sustained at 70 ± 10 Hz to maintain a constant workload of 80 W, equivalent to a modestly intense riding speed of 3.8 $m \cdot s^{-1}$ (8.5 miles/h) [22]. Exercise was sustained for 20 min, followed by a 20 min recovery period during which subjects rested while seated. Study visits were performed 1 week apart at the same time of day on each visit.

A venous blood sample (5 ml) was collected in serum gel tubes (Sarstedt, Leicester, U.K.), via the non-infused forearm cannula, for measurement of serum UA and total serum antioxidant capacity at baseline and 20, 40 and 60 min after the start of the infusion. Additional 5 ml samples were collected in potassium/EDTA tubes (Sarstedt) for determination of plasma 8-iso-$PGF_{2\alpha}$ concentrations at baseline and 60 min after the start of infusion. Blood samples were centrifuged at 1000 *g* for 10 min at 4 °C, decanted immediately, and serum and plasma were stored at −40 °C until assays were performed.

Haemodynamic variables were determined at baseline and at 5 min intervals up to 60 min after the start of the infusion. BP was recorded in the dominant arm using a validated oscillometric device (HEM-705CP; Omron, Tokyo, Japan) [23], and the cardiac index (CI) was assessed using transthoracic bioimpedance (NCCOM3-R7; BoMed, Irvine, CA, U.S.A.) [24]. Systemic vascular resistance index was calculated as mean arterial pressure divided by CI.

Serum UA concentration

UA concentration was determined by an automated colorimetric assay (Vitros; Ortho-Clinical Diagnostics, Amersham, Bucks, U.K.).

Serum antioxidant capacity

Antioxidant capacity was measured using the Total Antioxidant Status assay (Randox Laboratories; Crumlin, County Antrim, U.K.). This assay is based on the interaction between a chromogen [2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)] and ferrylmyoglobin, a free radical formed by the reaction of metmyoglobin and H_2O_2, which forms the cation $ABTS^{\bullet+}$, a blue/green chromophore with maximal absorbance at 417, 645, 734 and 815 nm [25]. Antioxidants in added serum scavenge $ABTS^{\bullet+}$ and prevent absorbance to a degree related to the overall serum antioxidant capacity. Absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, Lewes, East Sussex, U.K.), calibrated using 6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid (Trolox, a water soluble tocopherol analogue) and expressed as μmol/l of Trolox equivalent. The Total Antioxidant Status assay gives a global measurement of *ex vivo* serum free-radical-scavenging capacity and takes account of summation and interaction between constituent antioxidants [25]. The standard reference range for this assay was 1494–2107 μmol/l and the intra-assay precision was 6.5 %.

Plasma 8-iso-PGF_{2a} concentrations

Solid-phase extraction was performed using an Isosolute C18(EC) 100 mg/3 ml silica-sorbent column (International Sorbent Technology, Hengoed, Mid Glamorgan, U.K.), and 8-iso-$PGF_{2\alpha}$ was eluted with ethylacetate/methanol (99:1, v/v) buffer. Assays were performed on undiluted samples and after 1:2 dilution with buffer, and each concentration was assayed in duplicate. 8-iso-$PGF_{2\alpha}$ concentrations were measured using an ELISA (Cayman Chemical Co., Ann Arbor, MI, U.S.A.). The assay is based on the competition between 8-iso-$PGF_{2\alpha}$ and an acetylcholinesterase–8-iso-$PGF_{2\alpha}$ conjugate for limited amounts of 8-iso-$PGF_{2\alpha}$-specific rabbit IgG bound to 96-well plates [3]. Ellman's reagent [5,5′-dithiobis-(2-nitrobenzoic acid)] contains an acetylcholinesterase substrate, which forms a distinctive yellow reaction product whose absorbance was detected at 412 nm using a Vmax kinetic microplate spectrophotometer (Molecular Devices, Winnersh, U.K.). The extent of absorbance is proportional to the amount of conjugate in each well, which is inversely proportional to free 8-iso-$PGF_{2\alpha}$ concentrations during incubation. Mean values were expressed as a percentage of maximal binding absorbance, and 8-iso-$PGF_{2\alpha}$ content was determined by comparison with absorbance of standard isoprostane concentrations (3.9, 7.8, 15.6, 31.3, 62.5, 125, 250 and 500 pg/ml). The standard reference range was 4.6–65.9 pg/ml and the intra-assay precision was 6.0 %.

Table 1 Baseline characteristics of the study population

Values are means ± S.D.

Characteristic	Value
Number (male)	20 (10)
Age (years)	23 ± 3
Height (m)	1.72 ± 0.34
Weight (kg)	71 ± 5
Body mass index (kg/m^2)	23.0 ± 3.5
Serum creatinine (μmol/l)	72 ± 10
Serum cholesterol (mmol/l)	4.0 ± 2.5
Serum urate (μmol/l)	293 ± 52

Data analysis and statistics

Responses were compared using two-way ANOVA and paired Student's *t* tests where appropriate, and statistical significance was accepted at the 5 % level in all cases.

RESULTS

Baseline characteristics of study subjects are shown in Table 1. As expected, UA administration caused serum concentrations to increase by 194 ± 8 μmol/l ($P < 0.001$) from baseline (Table 2). Administration of vehicle alone did not significantly change circulating UA concentrations from baseline (−9 ± 2 μmol/l). There was a corresponding increase in antioxidant capacity from baseline of 118 ± 18 μmol/l ($P < 0.001$) following UA

Table 2 Serum UA concentration, antioxidant capacity and haemodynamic variables at baseline, after infusion of 0.5 g of UA in 250 ml of vehicle or vehicle alone, after exercise and after recovery

Values are means ± S.E.M. *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$ compared with vehicle alone. SVRI, systemic vasculature resistance index.

	Tretment	Baseline (0 min)	Post-infusion (20 min)	Post-exercise (40 min)	Post-recovery (60 min)
Serum urate (μmol/l)	Vehicle	294 ± 19	286 ± 18	296 ± 19	293 ± 18
	UA	293 ± 16	487 ± 16***	458 ± 15***	429 ± 14***
Antioxidant capacity (μmol/l)	Vehicle	1815 ± 29	1769 ± 46	1817 ± 27	1796 ± 32
	UA	1786 ± 39	1899 ± 45**	1937 ± 44*	1895 ± 47*
Heart rate (beats per min)	Vehicle	75 ± 2	73 ± 2	138 ± 6*	80 ± 2
	UA	76 ± 3	74 ± 2	135 ± 7*	80 ± 3
Systolic BP (mmHg)	Vehicle	93 ± 5	97 ± 6	147 ± 6*	94 ± 4
	UA	94 ± 5	94 ± 5	153 ± 6*	97 ± 5
Diastolic BP (mmHg)	Vehicle	66 ± 2	67 ± 3	101 ± 5*	69 ± 2
	UA	68 ± 2	66 ± 4	103 ± 6*	68 ± 2
Cardiac index (l/min per m^2)	Vehicle	3.9 ± 0.2	3.8 ± 0.2	9.7 ± 0.8*	3.8 ± 0.2
	UA	3.9 ± 0.2	3.7 ± 0.1	9.4 ± 0.6*	4.0 ± 0.2
SVRI	Vehicle	22.0 ± 2.0	23.2 ± 1.7	15.9 ± 2.4*	22.0 ± 1.7
	UA	22.4 ± 1.9	23.6 ± 1.6	14.5 ± 1.2*	23.0 ± 2.0

administration, but no change following vehicle alone (− 22 ± 23 μmol/l).

Plasma 8-iso-$PGF_{2\alpha}$ concentrations in the study population were 41.8 ± 3.0 pg/ml at baseline. Intense physical exercise caused a significant increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations from 35.0 ± 4.7 pg/ml to 45.6 ± 6.7 pg/ml immediately after exercise ($P < 0.05$), and 55.7 ± 7.1 pg/ml after recovery ($P < 0.01$) following vehicle administration (Figure 1). However, after systemic administration of UA, there was no significant increase from baseline plasma 8-iso-$PGF_{2\alpha}$ concentrations (37.1 ± 5.4 pg/ml) after exercise (31.6 ± 5.5 pg/ml) or after recovery (37.9 ± 6.6 pg/ml). Plasma 8-iso-$PGF_{2\alpha}$ concentrations after vehicle and UA administration, expressed as a change from baseline, were + 10.6 ± 5.5 and − 5.5 ± 6.2 pg/ml ($P < 0.05$) respectively, after exercise, and + 20.7 ± 6.5 and + 0.8 ± 7.7 pg/ml ($P < 0.01$) respectively, after recovery. Two-way ANOVA showed a significant effect of UA administration on isoprostane concentrations ($P = 0.02$) and changes in isoprostane concentrations from baseline ($P < 0.01$) during exercise and recovery.

Administration of neither vehicle nor UA caused any significant changes in resting haemodynamic variables from baseline (Table 2). Intense physical exercise caused significant increases in heart rate ($P < 0.001$), systolic BP ($P < 0.001$), diastolic BP ($P < 0.001$) and CI ($P < 0.005$), and a reduction in systemic vascular resistance index ($P < 0.005$), whereas administration of UA did not influence the haemodynamic responses. No adverse events were reported in any of the subjects.

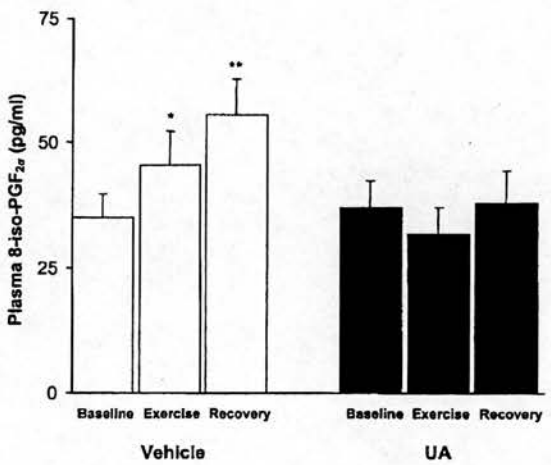

Figure 1 Plasma 8-iso-$PGF_{2\alpha}$ concentrations at baseline, after acute physical exercise and after recovery following administration of 0.5 g of UA in 250 ml of vehicle or vehicle alone

Values are means ± S.E.M. *$P < 0.05$ and **$P < 0.01$ compared with baseline.

DISCUSSION

In the present study, the baseline characteristics were consistent with those of a young healthy population free from major cardiovascular risk factors that could potentially exert independent effects on antioxidant capacity and oxidative stress. The chosen exercise intensity and duration caused a significant haemodynamic response in

this group, who were unaccustomed to regular strenuous exercise. Resting plasma free 8-iso-$PGF_{2\alpha}$ concentrations were consistent with those reported previously [26] in healthy subjects. The single bout of acute intense exercise caused oxidative stress in the study population, reflected by increased plasma 8-iso-$PGF_{2\alpha}$ concentrations immediately after exercise and recovery. The magnitude of the rise in plasma isoprostane concentrations was similar to previous observations in healthy subjects and trained athletes after acute exercise [27].

Vehicle administration had no significant effect on circulating UA concentrations or serum antioxidant capacity, as found previously [20]. Administration of UA (0.5 g) achieved a substantial increase in circulating UA concentrations to levels characteristic of the upper limit of the normal reference range (120–420 μmol/l). Raised UA concentrations were associated with increased serum free-radical-scavenging capacity, as indicated by a global antioxidant measurement. Prior administration of UA attenuated the exercise-induced increase in plasma 8-iso-$PGF_{2\alpha}$ concentrations. It is likely that this effect was mediated by the increased ability to counter excess free radical activity, conferred by the antioxidant properties of UA. These findings indicate that high circulating UA concentrations are able to prevent oxidative stress *in vivo* during intense physical exercise and raise the possibility that UA could protect against oxidative stress in other situations. Interestingly, a number of earlier observations suggest that local UA concentrations may be regulated, at least in part, by prevailing redox conditions. For example, oral administration of L-arginine has been found to cause a reduction in systemic oxidative stress, which is accompanied by a fall in circulating UA concentrations [28]. Furthermore, acute ischaemia in several vascular beds, for example the coronary circulation, is associated with oxidative stress and causes an increase in circulating UA concentrations locally [29,30]. Raised UA concentrations could, therefore, provide a physiological mechanism to protect against excess free radical activity.

The lack of effect of vehicle or UA administration on resting BP and systemic vascular resistance indicate that high UA concentrations, at least in the acute setting, do not directly influence vascular tone.

Cardiovascular risk and the development of atherosclerosis are likely to be influenced by exposure to chronic oxidative stress. A limitation of the present study is that only short-term measures of antioxidant capacity and oxidative stress were examined. However, this appears to be a valid approach because there is good correlation between short-term and long-term effects of a variety of cardiovascular risk factors on oxidative stress, for example cigarette smoking [31] and hyperglycaemia [32]. A further potential limitation is that the validity of the Omron HEM-705CP device has not been established in exercising subjects. It is accurate for measuring BP up to 160/100 mmHg in resting subjects and has been validated according to criteria of the British Hypertension Society and Association for the Advancement of Medical Instrumentation [23].

In conclusion, the present study has shown that UA administration temporarily raises circulating UA concentrations, which increases serum antioxidant capacity and reduces exercise-induced oxidative stress in a young healthy population. These findings indicate that the antioxidant properties of UA are of physiological consequence and support the view that UA confers potentially important free-radical-scavenging effects *in vivo*. The implications of raised serum UA concentrations, in the setting of chronic oxidative stress, require further evaluation. Additional research is required to investigate the potential role of high UA concentrations in patients exposed to major cardiovascular risk factors.

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Commentary

QJM

Uric acid: an important antioxidant in acute ischaemic stroke

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Introduction

An association between raised serum uric acid (UA) concentration and increased cardiovascular risk has been recognized for over 50 years.[1] A number of major epidemiological studies have identified high UA concentrations as an important risk marker for stroke in unselected populations. Furthermore, raised serum UA concentrations are associated with increased risk of stroke in high risk patient groups, for example those with hypertension or type 2 diabetes mellitus.[2,3] However, the significance of these relationships remains subject to considerable debate. Both *in vitro* and *in vivo* studies have shown UA to be a powerful free radical scavenger in humans and, paradoxically, these antioxidant properties could be expected to offer a number of benefits within the cardiovascular system.[4] No potential biological mechanisms are known by which raised UA concentrations could influence the development of stroke. Therefore, it is unclear whether high UA concentrations promote or protect against the development of cardiovascular disease, or simply act as a passive marker of increased risk. Not only has there been speculation surrounding the possible effects of UA on development of atherosclerosis but, over recent years, increasing attention has been paid to its potential role in the disease manifestations that ensue. In particular, emerging evidence suggests that UA plays an important role in acute ischaemic stroke, as a consequence of its antioxidant properties.

Antioxidants and stroke

Cerebral infarction initiates a complex cascade of metabolic events in the surrounding tissue, and free-radical-mediated oxidative damage plays a key role in the pathogenesis of cerebral ischaemia.[5] Free radicals are liberated from a variety of sources, including inflammatory cells, dysfunctional mitochondria and excitotoxic mechanisms stimulated by increased glutamate and aspartate concentrations.[6] Hydroxyl radicals (formed from hydrogen peroxide) peroxynitrite and superoxide are powerful radicals that can cause lipid peroxidation, a self-propagating chain reaction, that irreversibly damages plasma and mitochondrial membranes.[7] Products of lipid peroxidation, for example malondialdehyde, irreversibly disrupt enzymes, receptors, and membrane transport mechanisms. In acute ischaemic stroke, *in vivo* concentrations of lipid peroxidation products are significantly increased, arising from excess free radical activity (Figure 1).[8] Plasma concentrations of cholesteryl ester hydroperoxides (CEOOH) are sensitive and specific markers of lipid peroxidation, and correlate positively with infarct volume, calculated by computed tomography, and clinical severity, determined by the National Institute of Health Stroke Scale.[8] This emphasizes the role of oxidative stress in mediating cerebral ischaemic tissue damage, and is consistent with the observation that stroke volume is greater in patients with diminished antioxidant capacity.[9] These observations have stimulated interest in the possibility that

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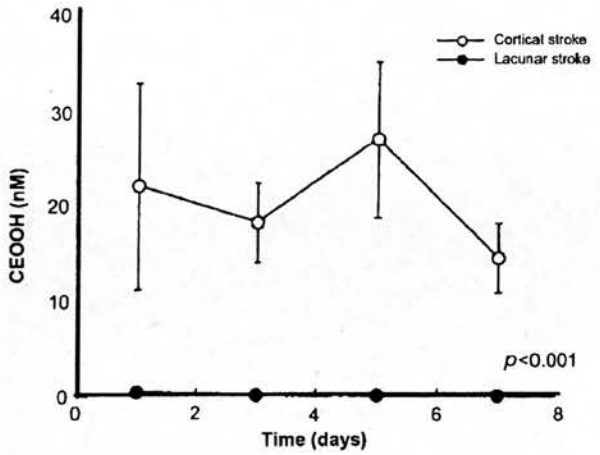

Figure 1. Mean ± SEM plasma cholesterol ester hydroperoxide (CEOOH) concentrations, an index of lipid peroxidation, in patients with large artery cortical stroke ($n = 32$), and age-matched patients with lacunar stroke ($n = 13$); $p < 0.05$ by two-way ANOVA. Reprinted from reference 8 with permission from Elsevier Science.

antioxidant treatments could offer benefits in acute ischaemic stroke, through their ability to defend against excess free radical activity.

Uric acid

UA is the most abundant aqueous antioxidant in humans, and contributes as much as two-thirds of all free radical scavenging capacity in plasma. It is particularly effective in quenching hydroxyl, superoxide and peroxynitrite radicals, and may serve a protective physiological role by preventing lipid peroxidation.[10] In a variety of organs and vascular beds, local UA concentrations increase during acute oxidative stress and ischaemia, and the increased concentrations might be a compensatory mechanism that confers protection against increased free radical activity.[4] In animal models, local UA concentrations significantly increase in acute brain injury (Figure 2).[11] For example, in the rat, middle cerebral artery occlusion causes a significant increase in cerebral UA concentrations, which can persist for several days after the injury.[12] These observations have prompted interest in the potential impact of raised local UA concentrations in the setting of acute ischaemic stroke.

Models of ischaemic neuronal injury have shown that the addition of physiological concentrations of UA protects hippocampal neurons against excitotoxic and metabolic injury *in vitro*.[13] The effects of raising circulating UA concentrations, by direct administration, have also been studied *in vivo* in a rat model of acute ischaemic stroke, involving

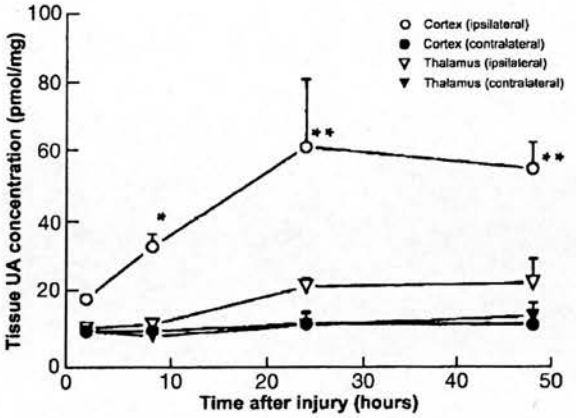

Figure 2. Mean ± SEM UA concentrations in cortex and thalamus at 0, 1, 8, 24, and 48 h after experimental brain injury in Wistar rats ($n = 4$–5 at each time point). Ipsilateral cortical and thalamic concentrations were higher than contralateral concentrations; $p < 0.0001$ by ANOVA for both, $^{*}p < 0.01$, $^{**}p < 0.001$ using Bonferroni two-tailed tests. Reprinted from reference 11 with permission from Elsevier Science.

transient occlusion of one middle cerebral artery for 2 h. Administration of UA, prior to ischaemia or during the subsequent reperfusion period, caused a significant reduction in infarct volume, and led to improved behavioural outcome at 24 h (Figure 3).[13] These findings suggest that early elevation of UA, during or shortly after acute ischaemic stroke, could confer significant protection against neurological deficit. This is consistent with the protective effects of UA observed in other models of cerebral diseases mediated by free radicals.

A recent study lends support to this hypothesis in a clinical setting. Serum UA concentrations measured in 881 consecutive ischaemic stroke patients at the onset of ischaemic symptoms were found to correlate inversely with early neurological impairment and final infarction size on computed tomography or magnetic resonance imaging.[14] Additionally, serum UA concentrations were positively associated with a good clinical outcome at hospital discharge (Matthew score of >75), where each mg/dl UA increase (equivalent to 60 μmol/l; reference range 120–420 μmol/l) was associated with a 12% increase in the odds of a good outcome. Importantly, these relationships were independent of potential confounders, including age, diuretic use, renal function or the presence of major cardiovascular risk factors. This is the first study to characterize the relationship between serum UA concentration and neurological severity of acute ischaemic stroke in a large series of patients. A potential limitation of this observational data is that it does not directly address the potential mechanisms by which UA could improve stroke

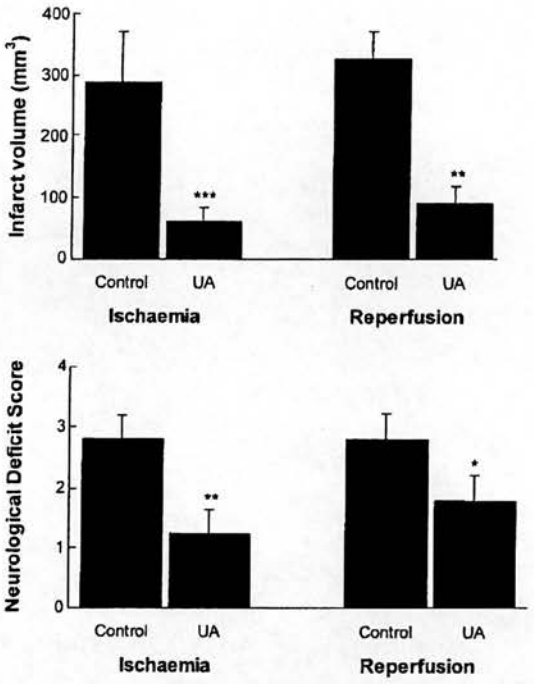

Figure 3. Mean ± SEM infarct volume and behavioural deficits 24 h after middle cerebral artery occlusion for 2 h in male Sprague Dawley rats, showing the effects of UA or saline (control) administration prior to ischaemia or during reperfusion. $*p<0.05$, $**p<0.01$, $***p<0.001$, by paired Student's *t* tests. Reprinted from reference 13 with permission from Wiley.

outcome, for example measurements of antioxidant capacity or oxidative stress. However, its findings support the potential benefits of raised UA concentrations observed in *in vitro* and *in vivo* experimental models.

Despite the widely held view that elevated serum UA concentrations confer increased risk of atherosclerotic disease, there is no compelling biological evidence of a causal link. Free radical activity is characteristically increased in patients with any one of several major cardiovascular risk factors, and is thought to play a key role in the early development of atherosclerosis. As an antioxidant, UA could be expected to confer protection against free radicals. In the context of acute ischaemic stroke, there is growing evidence to support a protective role for UA. This underpins the importance of oxidative stress in the pathogenesis of acute stroke, and strengthens the rationale for further investigation of antioxidant treatments in this condition. The feasibility of UA administration to temporarily increase circulating concentrations has recently been established,[15] and might allow its potential therapeutic impact to be examined in a clinical setting. Ongoing basic research is likely to shed new light on the cardiovascular effects of UA, and will hopefully allow the significance of serum concentrations to be interpreted more clearly.

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The authors observe that mortality from the combined effects of systolic blood pressure and serum cholesterol although higher in men than women, is not supported by any definite cause. We would like to offer the following suggestions.

The use of hormone replacement therapy (HRT) in women in the age group 45–55 has become common. It is also well known that this therapy raises high density lipoprotein cholesterol levels and lowers low density lipoprotein cholesterol levels. Therefore, the possibility of exogenous/endogenous oestrogen playing a role in the lower mortality rate observed in women cannot be excluded. Maybe if the authors analysed the number of women on HRT, our suggestion might be substantiated.

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Uric acid concentrations and the mechanisms of cardiovascular disease

We read with interest the article by Yusuf and Bosch[1], which addresses the potential role of uric acid (UA) as a causal factor in cardiovascular disease. The association between raised serum UA concentrations and increased cardiovascular risk is unequivocal, but observational or epidemiological data are unlikely to resolve whether UA is a causal, compensatory, or co-incidental factor. We support the authors' view that basic and experimental studies are now needed to identify potential biological links between UA and mechanisms of atherosclerosis. We also agree that allopurinol may have therapeutic potential in this setting. However, we are not convinced that the proposal of the authors, to use allopurinol to lower serum UA concentrations, will effectively address the mechanistic issues.

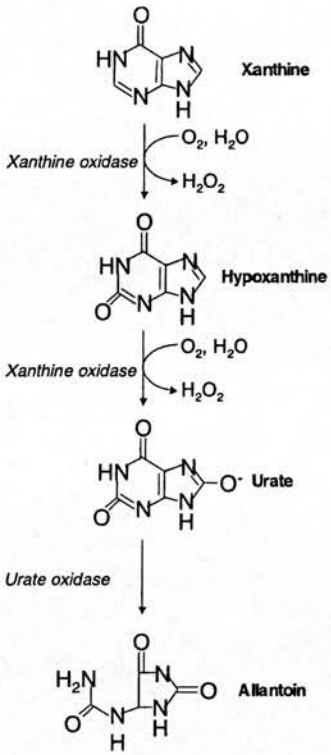

Figure 1 **Uric acid metabolism.**

Allopurinol causes a modest reduction in serum UA concentration, up to 30%[2], by inhibiting xanthine oxidase (XO), which normally catalyses conversion of hypoxanthine to xanthine, and xanthine to UA, an end product of purine metabolism in man (Fig. 1). However, XO activity also results in formation of hydrogen peroxide (H_2O_2), a potentially detrimental free radical, which is thought to contribute to vascular dysfunction, for example in patients with hypertension. In Dahl hypertensive rats, endothelial XO activity makes an important contribution to oxidative stress[3]. In humans, hypertensive patients have significantly greater H_2O_2 production than normotensive individuals, and the consequent increase in oxidative stress raises blood pressure and promotes target organ damage[4]. Inhibition of XO significantly reduces H_2O_2 production, and ameliorates vascular oxidative stress.

Additionally, allopurinol has antioxidant properties, which are independent of its effects on XO activity[5]. The potential cardiovascular effects of allopurinol could be due to inhibition of XO-mediated free radical generation, or direct antioxidant quenching of free-radical activity, and its use as a urate lowering agent requires cautious interpretation. The potential clinical benefits of allopurinol are threefold, but do not specifically address the question of a biological link between UA and mechanisms of atherosclerosis.

Another approach to lowering serum UA concentrations is administration of the enzyme urate oxidase (UO), which catalyses the further metabolism of UA. UO substantially lowers circulating UA concentrations, by up to 90%[6], and restoration can take several days, depending on the rate of purine metabolism. UO provides an opportunity to study the direct cardiovascular effects of UA lowering, in the absence of effects on XO or H_2O_2 liberation. Therefore, UO is a more effective and specific means of lowering serum UA concentrations than allopurinol and may, therefore, provide better insight into the relationship between UA and mechanisms of atherosclerosis.

An alternative approach to UA lowering is to study the cardiovascular effects of raising circulating UA concentrations, and the feasibility of systemic UA administration has recently been established[7]. This is an equally valid approach, because acute elevation of established major cardiovascular risk factors has been shown to cause impaired endothelial function in healthy individuals, for example after ingestion of a meal rich in saturated fats[8], or after raising circulating homocysteine concentrations by oral methionine administration[9].

We accept that allopurinol may confer significant cardiovascular benefits, and its therapeutic potential should be explored. However, the short-term approaches we have outlined are more likely to allow a better understanding of the possible mechanisms that link raised serum UA concentrations and increased cardiovascular risk.

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Myocardiocyte loss due to apoptosis

The findings presented by Dispersyn *et al.* (June 2002 issue)[1] on dedifferentiation and apoptosis of myocardiocytes late after AMI were extremely interesting. The presence of both myocytes with metabolic rearrangement ('dedifferentiated' or 'hibernated') and cells committed to programmed death (apoptotic) in this experimental model of post-infarction left ventricular remodelling in sheep suggests that both these mechanisms are possible cellular responses to ischaemia.

However, a major issue is the effective myocardiocyte loss due to apoptosis. The data presented by the authors[1] on myocardial apoptosis substantially confirm results presented earlier in a similar model of multiple AMI in dogs[2] (0·12% of apoptosis in the former and 0·53% in the latter), while they appear strikingly different from our recent data in post-mortem human hearts[3]. We have recently shown that the apoptotic rate is as high as 25% in the infarcted area of the hearts of human subjects who died 12 to 62 days after an AMI[3]. A comparison of the two studies reveals a nearly 200-fold increase in our samples compared to the data by Dispersyn *et al.*[1]. Could these differences be due to selection biases in both studies? Perhaps yes.

We believe that estimates of apoptotic rates may show quite different results in hearts of individuals dying spontaneously compared to animals surviving and being killed. In facts, our results[3] may have been biased by the selection of a population with a significantly poor prognosis (median time to death 23 days), who may have been associated with extremely elevated rates of apoptosis. On the other hand, Dispersyn *et al.*[1] may have selected individuals who were relatively protected from apoptosis with a more favourable prognosis (the sheep with a 50% reduction in left ventricular ejection fraction were allowed to survive at least 6 weeks after the last embolization).

We think it would be of interest to the readers of this Journal to know whether any of the sheep in the protocol by Dispersyn *et al.*[1] died before reaching the time they were due to be killed (as reported for the similar protocol in dogs[4]). If this were the case, it would be extremely interesting to obtain data regarding the apoptotic rates in this group of animals, who were characterized by a certainly more unfavourable prognosis.

Most experimental studies in animals are affected by a similar selection bias. Sabbah *et al.*[4] reported that approximately 30% of their dogs died early after intervention and were therefore excluded from the study. On the other hand, when, in an experimental study conducted on mice[5], all individuals were followed from time of intervention (surgical constriction of the aorta) until death, these animals, apoptosis-prone due to genetic manipulation, had an apoptotic rate greater than 30% and almost all died of dilated cardiopathy within a few weeks. Furthermore, a human observational study of post-mortem samples showed an apoptotic rate of 11·6% up to 10 days after AMI[6].

We were unable to understand, moreover, whether coronary embolization in the protocol by Dispersyn *et al.*[1] resulted in total occlusion of a major coronary artery. In particular, we would like to know if coronary artery angiograms were repeated before they were killed. Patency of the infarct-related artery at the time of death may represent a major determinant for apoptosis late after AMI. We have shown that individuals with an open artery at the time of death have significantly lower apoptotic rates at sites of infarction[3].

An accurate definition of the factors promoting death or survival should lead to a more complete understanding of the variability observed in experimental animal models and human observational studies. Indeed it is possible that some individuals are relatively protected from apoptosis, surviving longer after AMI and showing only low grade apoptosis and a very low rate of transition from metabolic rearrangements to commitment to death, as in the cases presented by Dispersyn *et al.*[1].

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PHARMACOKINETICS AND DISPOSITION

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Lithium carbonate as a potential pharmacological vehicle: intravenous kinetics of single-dose administration in healthy subjects

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Objective: We have been developing lithium carbonate solution as a vehicle for delivery of uric acid in a research setting. We wished to determine the pharmacokinetics of a single systemic administration of 500 mg lithium carbonate (13.5 mmol free Li^+) in healthy subjects.
Methods: Ten healthy subjects received 500 ml of a 0.1% lithium carbonate and 4% dextrose solution intravenously over 1 h. Serum lithium concentrations were determined at baseline, 15, 30, 45, 60, 75, and 90 min, and 2, 3, 7, 24, and 48 h after the start of infusion for kinetic analysis.
Results: Administration led to a time-dependent increase in plasma concentration, followed by a rapid decay of serum lithium concentration. Kinetic analysis showed that the pattern best fit a two-compartment model, with rapid extravascular distribution, an elimination phase half-life of 7.8 ± 1.7 h, and clearance of 5.3 ± 1.1 l/h.
Conclusions: In healthy subjects, lithium half-life is shorter and clearance is higher than suggested by previous reports in other groups. Administration of 500 ml 0.1% lithium carbonate and 4% dextrose over 1 h is safe, well tolerated, and possibly a suitable vehicle for other agents such as uric acid.

Keywords Lithium carbonate · Solubility · Two-compartment decay · Uric acid

We declare that the studies performed comply with current United Kingdom laws.

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Introduction

Lithium carbonate was first introduced into medical practice in the late nineteenth century for dissolution of bladder stones and rheumatic nodules, in doses ranging from 111 mg to 1,034 mg (3–26 mmol Li^+) per day [1]. More than 50 years ago, lithium carbonate was found to be effective in psychiatric illness at doses of around 1,847 mg (50 mmol Li^+) per day [2], and lithium salts remain widely used in the treatment of bipolar affective disorder. Based on the theory that uric acid dissolves readily in lithium-based solutions, low-dose treatment was advocated for the prophylaxis of gout by several proponents, including James Parkinson [3], for many years. We sought to develop a lithium carbonate-based solution that would allow us to administer urate in a research setting in order to explore its known antioxidant properties. We determined that 1,000 mg uric acid could be dissolved readily in 500 ml of a 0.1% lithium carbonate and 4% dextrose vehicle, which could be administered by systemic infusion over 1 h. Lithium has a narrow therapeutic range (serum concentration 0.4–1.5 mmol/l), and toxicity becomes increasingly likely beyond 2 mmol/l, typically manifesting as apathy, tremor, restlessness, and in severe cases ataxia, renal dysfunction, seizures, coma, and death [4]. Lithium is not subject to protein binding and is cleared exclusively by renal excretion [5]. However, despite the use of lithium over many years, there is a surprising lack of consistent information about its clearance after administration of a single dose. Reported plasma elimination half-life after oral administration varies between 10 h and 50 h [6], and this variability may partly reflect delayed absorption from the gastrointestinal tract, which can occur over 3–6 h [5]. To determine its suitability as a vehicle for uric acid delivery, we studied lithium kinetics and cardiovascular responses associated with intravenous administration of 500 ml 0.1% lithium carbonate and 4% dextrose over 1 h to ensure that unacceptably high plasma concentrations and adverse effects would not be encountered.

Materials and methods

Subjects

Subjects were selected from the healthy volunteer database at the Clinical Research Centre of the University of Edinburgh and invited to participate. Included were males and females aged 18 to 40 years, and written, informed consent was required before study participation. Exclusion criteria were clinical history of any major cardiorespiratory or renal disease, baseline serum creatinine concentration of >100 μmol/l, use of any medication, or pregnancy determined by urinary human chorionic gonadotropin-based assay [7]. The study protocol was approved by the local Research Ethics Committee, and ten subjects were recruited.

Study protocol

The study was conducted in the Clinical Research Centre and, after bladder voiding, subjects were asked to lie recumbent in a relaxed, quiet, temperature-controlled room (22–24°C). Standard 22-gauge cannulae were inserted into a large antecubital vein in each forearm using aseptic technique and local anaesthesia. After resting for 20 min to establish baseline conditions, 500 mg lithium carbonate (13.5 mmol free Li^+) in 500 ml 4% dextrose was infused over 1 h via the cannula in the nondominant arm, and 5-ml venous blood samples were drawn via the other cannula at baseline and 15, 30, 45, 60, 75, 90, 120, and 180 min for measurement of lithium concentration using an automated colorimetric assay (Vitros dry slide) (Johnson and Johnson, Calif., USA) [8]. The venous cannulae were removed and subjects allowed to leave, returning for three further venous blood samples at 7, 24, and 48 h.

At 0, 15, 30, 45, 60, and 75 min, heart rate and blood pressure were recorded in the dominant arm using a validated noninvasive oscillometric device (HEM-705CP, Omron, Japan) [9], and cardiac index was assessed using transthoracic bioimpedance (NCCOM3-R7, BoMed, Calif., USA) [10]. Values were compared to baseline using paired Student's *t*-tests.

Determination of elimination half-life

Standard linear and semilogarithmic graphs representing serum lithium concentration vs time were plotted. Curve fitting by nonlinear regression analysis was performed using Prism software, version 3.0 for Windows (GraphPad, San Diego, Calif., USA) [11]. F tests were used to compare best-fit curves generated by one- and two-exponent analyses for each subject, and statistical significance was accepted at the 5% level.

Results

Ten subjects (four males) aged 24 ± 2 years (mean ± SEM) with baseline serum creatinine 79 ± 7, body mass index 23 ± 1, and systolic blood pressure (BP) 104 ± 3 mmHg were studied. Lithium carbonate infusion led to a time-dependent increase in serum lithium concentration, with a peak concentration of 0.93 ± 0.05 mmol/l (Fig. 1). This was followed by a rapid decay that appeared consistent with a one- or two-compartment model (Fig. 2). The unweighted decay plot data were subjected to exponential and biexponential analyses and compared to determine the best fit. The F value was >1.0 in favour of the two-compartment model for data from each subject. As a measure of distribution around the residuals, the sum of squares for

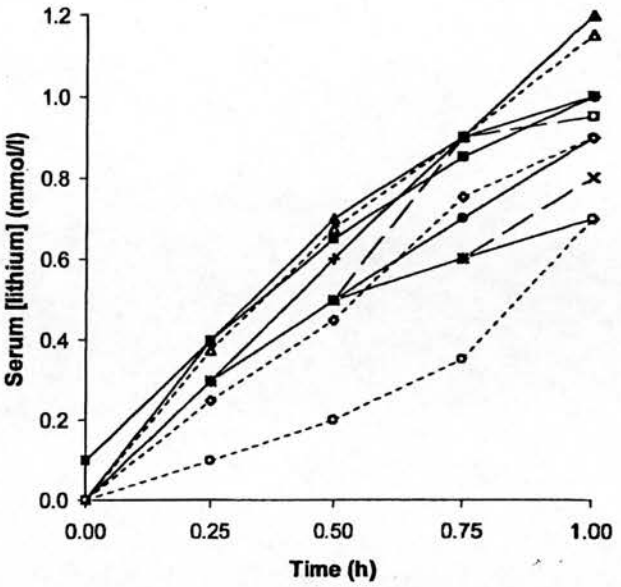

Fig. 1. Serum lithium concentration during intravenous administration of 500 mg lithium carbonate over 1 h in ten healthy subjects

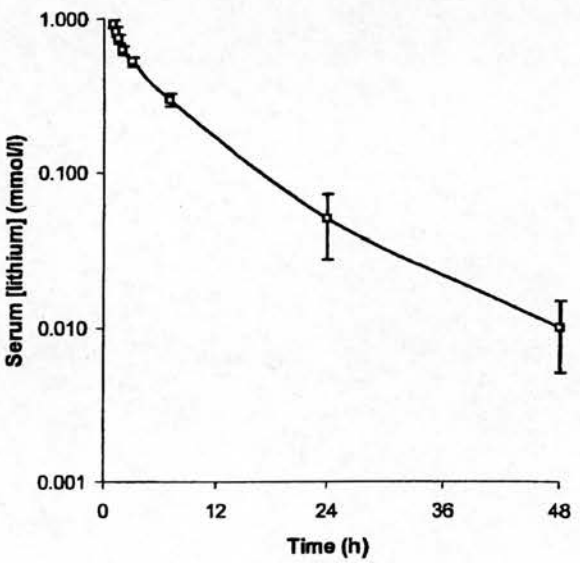

Fig. 2. Mean ± SEM serum lithium concentration after intravenous administration of 500 mg lithium carbonate over 1 h. $N=10$ (data at 48 h only available for six subjects)

the one- and two-compartment models were 1.313×10^{-3} and 7.37×10^{-6}, respectively ($F=96.06$ and $P=0.01$ for the averaged data). Nonlinear biexponential regression analysis of individual subject data found an elimination half-life of 7.8 ± 1.7 h, distribution phase half-life of 37 ± 12 min, volume of distribution of 43.4 ± 2.3 l, and clearance of 5.3 ± 1.1 l/h.

There were no significant effects on serum electrolyte or creatinine concentrations, and there was a transient increase in systolic BP, reduction in heart rate, and cardiac index (Table 1).

Table 1. Serum chemistries and systemic haemodynamics at baseline and after administration of 500 mg lithium carbonate over 1 h

	Baseline	1 h	4 h	24 h
Sodium (mmol/l)	140±1	139±1	140±1	141±1
Potassium (mmol/l)	3.8±0.2	3.8±0.3	3.9±0.2	4.0±0.2
Urea (mmol/l)	4.2±1.1	4.3±1.0	4.0±1.1	3.9±1.2
Creatinine (μmol/l)	79±7	78±6	77±6	77±7
Bicarbonate (mmol/l)	24±2	24±1	24±2	25±2
Uric acid (mmol/l)	0.23±0.05	0.23±0.05	0.22±0.05	0.23±0.05
Systolic BP (mmHg)	104±3	107±3*	–	–
Diastolic BP (mmHg)	64±2	66±2	–	–
Heart rate (bpm)	59±3	55±2*	–	–
Cardiac index (l/min per m^2)	4.2±0.2	3.8±0.2*	–	–

*$P < 0.05$ compared to baseline

Discussion

We observed lithium plasma kinetics following administration of a single intravenous dose in young healthy men and women with normal renal function. The peak serum lithium concentration of 0.93 mmol/l was towards the upper part of the normal therapeutic reference range (0.4–1.0 mmol/l) and followed by rapid extravascular distribution and progressive plasma clearance. We observed a decay of serum lithium concentration consistent with a two-compartment model, as previously reported [12, 13], and plasma elimination half-life was 7.8 h. Clearance was more rapid than suggested by previous observational studies of acute oral lithium intoxication [14] that may have been confounded by concomitant drug use, alcohol ingestion, or delayed systemic absorption as a consequence of impaired gastrointestinal motility following a high oral lithium load [15]. During steady-state maintenance treatment [16, 17] and acute toxicity superimposed on chronic treatment [2, 18], plasma clearance has also been found to be lower, possibly due to increased renal resorption in the presence of fluid balance disturbance and impaired renal tubular function due to drug toxicity [19].

The modest acute haemodynamic responses were consistent with the effects of a 500-ml intravenous fluid load [20] and unlikely to be of clinical significance.

A potential limitation of our present study is that all subjects were of a similar age and fewer than ideally required to develop a population kinetic model. Furthermore, elimination half-life calculations were based on comparatively infrequent sampling in the terminal phase. However, we were particularly interested in the peak lithium concentrations attained during systemic administration and the possible duration of exposure to high concentrations. Our findings indicate that the maximal concentrations attained by infusion of 500 mg lithium carbonate over 1 h are short-lived. Together with the rapid plasma clearance observed, we do not anticipate any significant risk of adverse effects.

In summary, intravenous administration of 500 mg lithium carbonate to healthy volunteers over 1 h results in transient exposure to moderate plasma concentrations. There was rapid plasma clearance, and no adverse effects were encountered. Therefore, 500 ml of a 0.1% lithium carbonate and 4% dextrose solution may provide a suitable vehicle for administration of compounds with limited solubility in conventional aqueous media in a research setting.

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Systemic Uric Acid Administration Increases Serum Antioxidant Capacity in Healthy Volunteers

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Summary: Oxidative stress plays an important role in the development of atherosclerosis and contributes to tissue damage that occurs as a consequence, particularly in myocardial infarction and acute stroke. Antioxidant properties of uric acid have long been recognized and, as a result of its comparatively high serum concentrations, it is the most abundant scavenger of free radicals in humans. Elevation of serum uric acid concentration occurs as a physiologic response to increased oxidative stress—for example, during acute exercise—thus providing a counter-regulatory increase in antioxidant defenses. In view of its antioxidant properties, uric acid may have potentially important and beneficial effects within the cardiovascular system. We wished to investigate whether administration of uric acid was feasible and if it could have an impact on antioxidant function in vivo. We have, therefore, performed a randomized, placebo-controlled double-blind study of the effects of systemic administration of uric acid, 1,000 mg, in healthy volunteers, compared with vitamin C, 1,000 mg. We observed a significant increase in serum free-radical scavenging capacity from baseline during uric acid and vitamin C infusion, using two methodologically distinct antioxidant assays. The effect of uric acid was substantially greater than that of vitamin C. **Key Words:** Urate—Antioxidant—Cardiovascular—Vitamin C—Chemiluminescence—Free radicals.

Uric acid is the final product of purine metabolism in humans, unlike the situation in virtually all other species, in which uric acid is further metabolized by the enzyme urate oxidase. The human urate oxidase gene has been located on chromosome 1, but is not expressed because of two nonsense mutations (1). Therefore, having lost the ability to express urate oxidase, humans are exposed to higher uric acid concentrations than other species, raising the possibility that these could confer an evolutionary advantage (2). Uric acid has important antioxidant properties in vitro, by scavenging free radicals (3) and chelating iron, the latter preventing iron-catalyzed oxidation (4). There is a strong correlation between the concentration of uric acid in biologic fluids and demonstrable antioxidant activity. Indeed, uric acid contributes as much as 60% of free-radical scavenging in human serum (5), and if persistently elevated uric acid concentrations offer an evolutionary advantage in humans, this may be because of its powerful antioxidant properties (3). Oxidative stress reflects an unfavorable imbalance between

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potentially harmful oxidants and protective antioxidants and predisposes to local tissue damage, partly mediated through excess free-radical activity (6–8). Oxidative stress plays an important role in the development of atherosclerosis (9), and acute oxidative stress occurs in ischemic stroke (10), acute lower limb ischemia (11), myocardial infarction (12), reperfusion injury (13), and acute physical exercise (14) as a result of tissue ischemia and altered cellular redox state. A consistent observation in these states is the subsequent elevation of serum uric acid concentration due to metabolism of adenosine generated by ischemic tissues (15–17), loss of the inhibition of xanthine oxidase caused by nitric oxide (18), and impaired oxidative metabolism (19). The observed uric acid elevation may be a protective response, capable of opposing the harmful effects of free-radical activity and oxidative stress. Smokers and patients with diabetes mellitus, hypertension, and hypercholesterolemia are exposed to chronic oxidative stress, which is thought to play an important role in the development and progression of atherosclerosis (20).

Each of these conditions is associated with an increased serum uric acid concentration, possibly conferring protection against persistent exposure to potentially damaging vascular oxidants, characteristically found in these conditions. There is further evidence that hydrophilic antioxidants play a pivotal role within the cardiovascular system; this evidence is underpinned by studies of the effects of vitamin C in humans. In diabetic subjects, hypertensive subjects, and those who use tobacco regularly, circulating vitamin C concentrations are lower (5,21,22). Diabetes, hypertension, and tobacco use are associated with characteristic impairment of nitric oxide–mediated endothelial function, which may be an important intermediary step in the development of atherosclerosis (23,24). Short-term administration of vitamin C to these groups leads to restoration of normal vascular function, offering further evidence that endothelial dysfunction may be a consequence of oxidative stress (22,25, 26). Furthermore, short-term administration of vitamin C, 2,000 mg, improves flow-mediated coronary vasodilatation in patients with coronary atherosclerosis, indicating that aqueous antioxidants cause functional improvement, even when vascular disease is already established (27).

Uric acid is a powerful free-radical scavenger, the serum concentration of which rises in the setting of acute and chronic oxidative stress. Its potential value as a therapeutic antioxidant has been recognized (28). We wished to explore whether high doses of uric acid could be administered systemically in humans, and to study its effects on circulating antioxidant capacity in direct comparison with vitamin C.

METHODS

Study volunteers

Eight healthy nonsmokers (three men and five women) aged 24 ± 2 years (mean age ± SEM) participated in these studies. Exclusion criteria included a history of renal, joint, or cardiovascular disease, use of any medication including antioxidant supplements, and pregnancy, which was determined by a human chorionic gonadotrophin–based urinary test.

Study protocol

The study protocol was reviewed and approved by the local research ethics committee and all participants provided written informed consent before participation. The study was randomized, placebo-controlled, and double-blind and used a crossover design involving 3 study days, each separated by at least 1 week. The study was conducted in a quiet, comfortable environment, maintained at 24°–26°C, within the Clinical Research Centre. Participants attended at 09:00 a.m. on each study day and were asked to avoid alcohol, caffeine, and purine-rich foods for 24 h before. An 18-G cannula was inserted into a large vein of each antecubital fossa using local anesthesia and aseptic technique. The cannula in the nondominant forearm allowed infusion over 60 min of 1,000 mg uric acid (5,952 μmol) in 500 ml 4% dextrose/0.1% lithium carbonate vehicle, vehicle alone, or 1,000 mg vitamin C (5682 μmol) in 500 ml 0.9% saline during separate visits, in a randomized order. Five milliliters of venous blood were drawn from the other cannula and collected in serum gel tubes (Sarstedt Ltd., Leicester, U.K.) at baseline and at 15-min intervals during infusion. Uric acid concentration was determined by an automated colorimetric assay (Vitros dry slide assay, Ortho-Clinical Diagnostics, Amersham, U.K.). Blood collected in a separate gel tube was allowed to clot, centrifuged at 6 *g* for 10 min at 4°C, decanted immediately, and stored at –40°C before determination of antioxidant capacity. Additional venous blood samples were drawn at 15, 30, 60, 120, 300, and 1,260 min after infusion for analysis of uric acid kinetics.

Determination of antioxidant capacity

The total antioxidant status (TAS) assay (Randox Laboratories, Antrim, U.K.) is based on the interaction between a chromogen (2,2′-amino-di-[3-ethylbenzthiazole sulfonate], ABTS) and ferrylmyoglobin, a free radical formed by the reaction of metmyoglobin and hydrogen peroxide (29). The resultant radical cation (ABTS·) is a blue-green chromophore with maximal absorbance at 417, 645, 734, and 815 nm (30). Antioxidants in added serum scavenge ABTS· and prevent ab-

sorbance to a degree related to overall serum antioxidant capacity. Absorbance was determined at 600 nm using a Cobas Fara (Roche Diagnostics, East Sussex, UK), calibrated using 6-hydroxy-2,5,8-tetramethylchroman-2-carboxylic acid, a water soluble tocopherol analogue (Trolox; Sigma, Dorset, U.K.), and expressed as m*M* of Trolox equivalent. The TAS assay gives an ex vivo quantification of global serum free-radical scavenging capacity and has been studied in a broad range of patients (29–32).

Luminol emits light in response to oxidation by hydrogen peroxide, catalyzed by horseradish peroxidase, detectable using a photocurrent device (33). Added serum containing radical-scavenging antioxidants delays photon emission due to consumption of intermediate radicals, and the resultant lag period is proportional to total serum antioxidant content (34). We used a luminometer (Model 1251; BioOrbit, Turku, Finland) to establish the lag period after addition of serum, calibrated to a standard curve generated by addition of 0.16, 0.32, 0.48, and 0.64 μ*M* Trolox, and serum antioxidant capacity was expressed as μ*M* of Trolox (35).

Data analysis

Dose-response relationships during infusion of uric acid and vitamin C were compared with those for vehicle using ANOVA. Where appropriate, values at each time point were compared using two-tailed Student's *t* tests, and statistical significance was accepted at the 5% level throughout.

RESULTS

Baseline serum uric acid and creatinine concentrations were 227 ± 9 μ*M* (normal range, 120–420 μ*M*) and 74 ± 4 μ*M* (normal range, 70–10 μ*M*), respectively. Infusion led to a significant time-dependent uric acid increment of 307 μ*M* ($p < 0.001$, compared with vehicle) (Fig. 1). TAS increased by 23% from baseline during uric acid infusion and by 7% during vitamin C infusion ($p < 0.001$ and $p = 0.65$, respectively, compared with vehicle) (Fig. 2). Antioxidant capacity determined by inhibition of chemiluminescence increased by 139% during uric acid infusion and 20% during vitamin C infusion ($p < 0.001$ and $p = 0.56$, respectively, compared with vehicle). Antioxidant capacity after uric acid infusion was significantly greater than after vitamin C and vehicle by both TAS and chemiluminescence-based assays ($p < 0.001$ in each comparison). Serum uric acid concentration exhibited a two-component decay, with mean elimination half-life of 10.8 hours (Fig. 3). No adverse effects of uric acid administration were encountered.

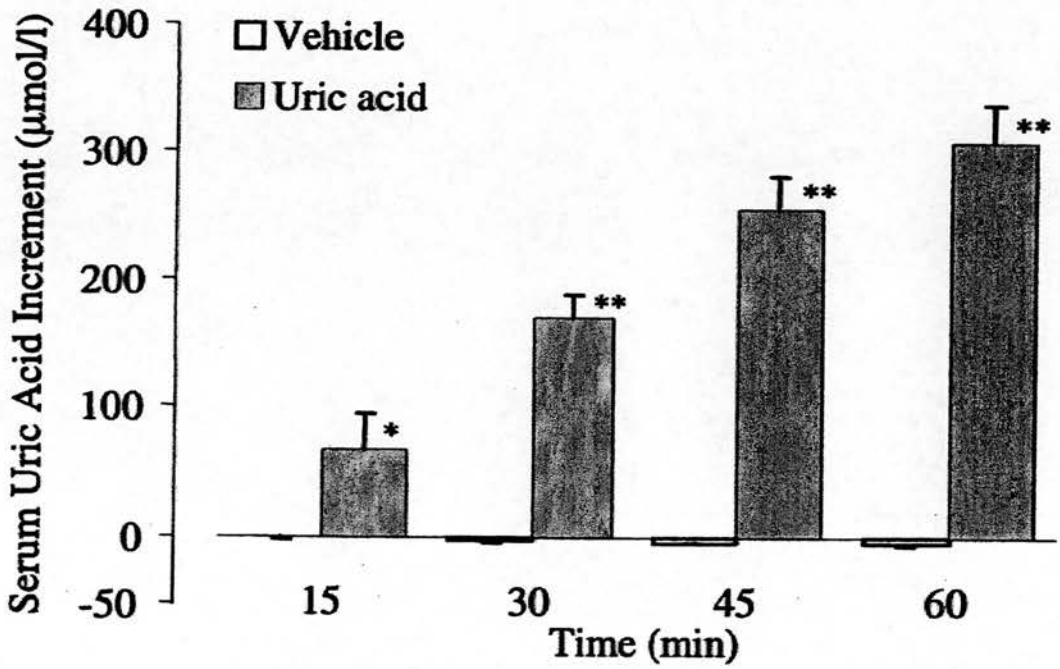

FIG. 1. Serum uric acid increment from baseline during infusion of 1,000 mg uric acid in 500 ml 4% dextrose/0.1% lithium carbonate vehicle or vehicle solution alone (mean ± SEM). $p < 0.001$ by ANOVA. *$p < 0.05$, **$p < 0.005$ using Student's two-tailed *t* test. Baseline concentration 227 ± 9 μ*M* (normal range, 120–420 μ*M*).

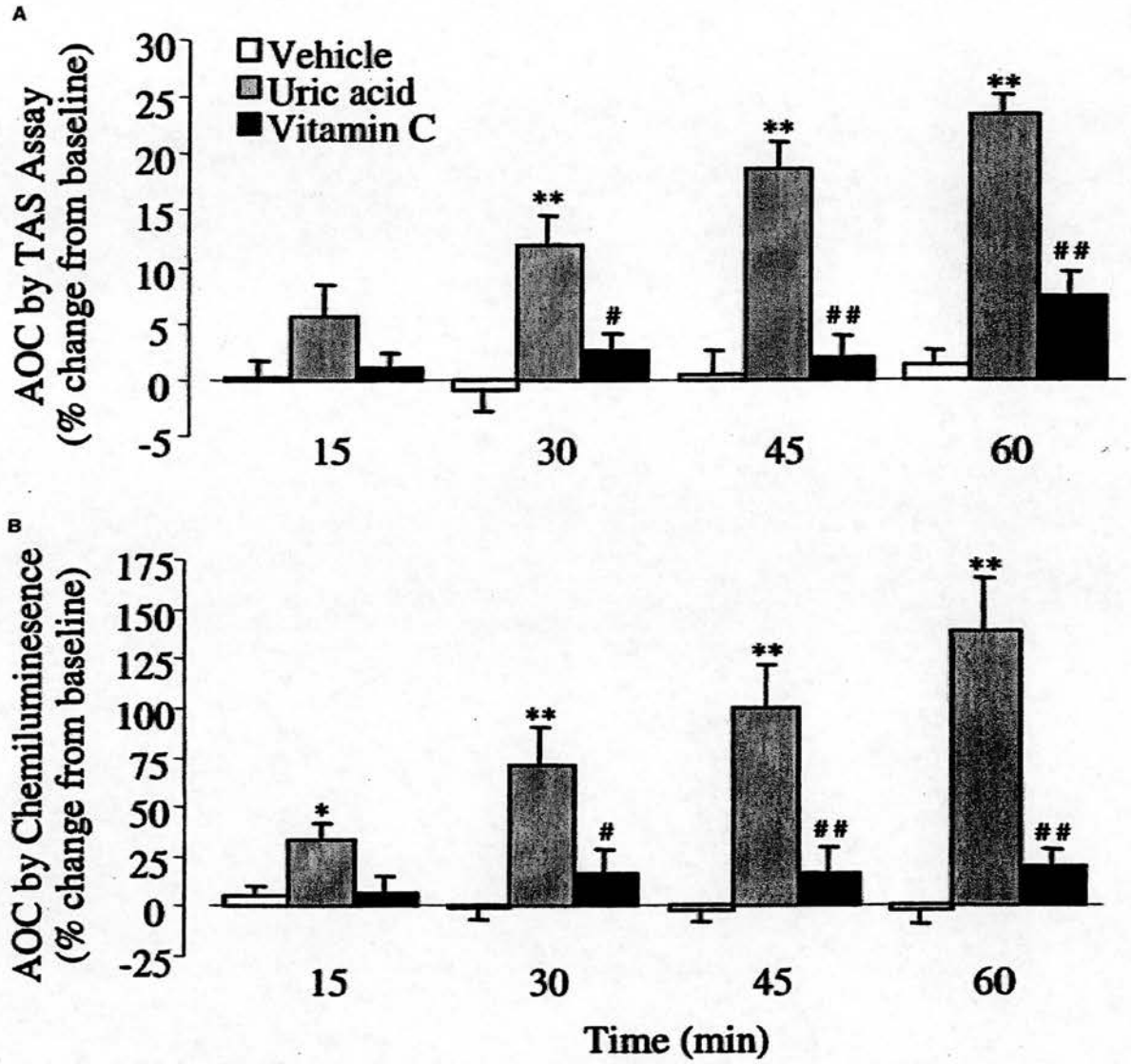

FIG. 2. Percentage change of serum antioxidant capacity (AOC) from baseline during infusion of 1,000 mg uric acid in 500 ml 4% dextrose/0.1% lithium carbonate, 500 ml vehicle alone, or 1,000 mg vitamin C in 500 ml 0.9% saline (mean ± SEM) determined by **(A)** total antioxidant status assay (Randox Laboratories, UK) and **(B)** inhibition of chemiluminescence. *$p < 0.01$, **$p < 0.005$ between uric acid and vehicle infusion, and #$p < 0.01$, ##$p < 0.005$ between uric acid and vitamin C infusion, using Student's two-tailed *t* tests.

DISCUSSION

We have previously described how a lithium-based vehicle can be used as an aqueous medium for pharmacologic delivery of uric acid (36). To the best of our knowledge, this study is the first to demonstrate the effects of uric acid administration on serum antioxidant capacity in humans. Uric acid administration substantially increases ex vivo antioxidant capacity in healthy volunteers with low baseline serum concentrations, consistent with normal antioxidant defenses and low oxidant stress (37). We anticipate that the impact of these effects may have been even greater in smokers or diabetic patients, who have low background antioxidant capacity. Serum uric acid is a potent free-radical scavenger and we have demonstrated, using two methodologically distinct assays, that systemic administration increases ex vivo serum free-radical scavenging capacity to a significantly

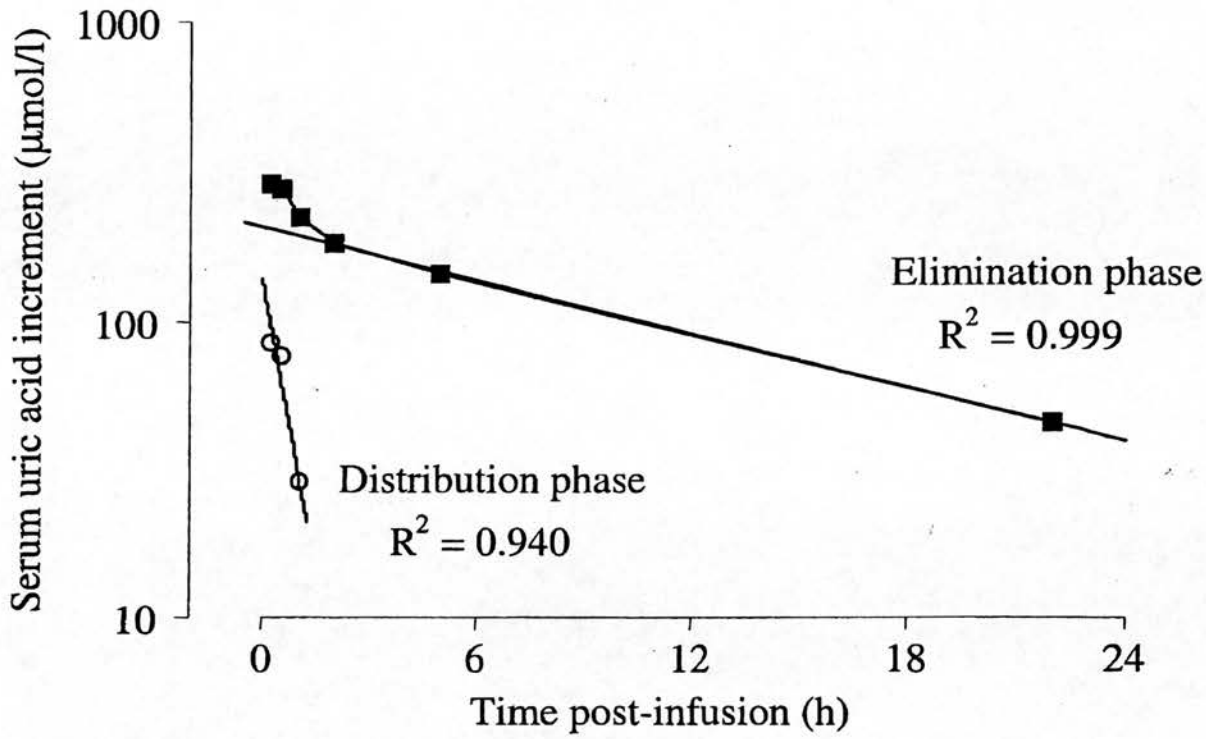

FIG. 3. Semi-logarithmic decay curve of serum uric acid increment (squares) after infusion of 1,000 mg of uric acid. Best-fit analysis of elimination phase gives $y = 209.7e^{-0.691x}$, and distribution phase (circles) by subtraction analysis gives $y = 139.2^{e-1.506x}$. Calculated elimination phase half-life = 10.8 h.

greater extent than vitamin C, another important aqueous physiologic antioxidant. It is possible, therefore, that the physiologic role of uric acid could be enhanced by short-term administration to prevent oxidative and free-radical-mediated tissue damage in, for example, sepsis syndrome, in which early administration of combined antioxidants led to significant improvement in cardiovascular hemodynamics (38). A further example of acute oxidative stress is seen in acute myocardial infarction, in which the subsequent rise of serum uric acid concentration can take several minutes or hours to reach maximal concentrations (39). However, systemic administration would have an immediate effect. The observed elimination half-life of administered uric acid suggests that the increase in antioxidant capacity is likely to persist for at least several hours after a single intravenous administration, which coincides with the period of maximal early free-radical activity, when potential for tissue protection is greatest (39,40). Early initiation of antioxidant treatment in myocardial infarction using combined vitamins A, C, and E and beta carotene led to a significant improvement in left ventricular systolic function and a trend toward overall mortality reduction (41). Repeated administration of uric acid may be unfavorable in view of the associations between persistent hyperuricemia and joint and renal disease, although it is difficult to determine an absolute serum concentration beyond which risk is significantly increased. In relation to joint disease, prolonged exposure to moderately elevated concentrations appears important because acute elevation, even to concentrations beyond 1,200 μM in tumor lysis syndrome, does not necessarily cause gout (42).

However, serum concentrations of this magnitude are associated with acute renal impairment due to precipitation of urate crystals in the collecting ducts and ureters, causing obstructive nephropathy. Paradoxically, renal impairment in the presence of chronic hyperuricemia is more often attributable to other factors, such as hypertension or diabetes, rather than serum uric acid concentration itself (43). We did not observe any adverse effects after administration of uric acid, 1,000 mg, in this study and we believe that the consequent short-term elevation of serum uric acid concentration poses a very low risk. The potential value of this novel therapeutic strategy requires further evaluation in a clinical setting.

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Review

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Uric acid as a risk factor for cardiovascular disease

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Introduction

Over recent years there has been renewed debate about the nature of the association between raised serum uric acid concentration and cardiovascular disease.[1] Several large studies have identified the value, in populations, of serum uric acid concentration in predicting the risk of cardiovascular events, such as myocardial infarction. This has directed research towards the potential mechanisms by which uric acid might have direct or indirect effects on the cardiovascular system. It has been difficult to identify the specific role of elevated serum uric acid because of its association with established cardiovascular risk factors such as hypertension, diabetes mellitus, hyperlipidaemia and obesity.[2,3] Indeed, it is not even clear at this stage whether uric acid has a damaging or protective effect in these circumstances. Increased understanding of the mechanisms underlying these associations may allow a clearer interpretation of the importance of elevated serum uric acid concentrations, and the potential value of specific urate-lowering treatment on cardiovascular disease.

Uric acid synthesis

Purines arise from metabolism of dietary and endogenous nucleic acids, and are degraded ultimately to uric acid in man, through the action of the enzyme xanthine oxidase (Figure 1). Uric acid is a weak acid (pKa 5.8), distributed throughout the extracellular fluid compartment as sodium urate, and cleared from the plasma by glomerular filtration.[4] Around 90% of filtered uric acid is reabsorbed from the proximal renal tubule, while active secretion into the distal tubule by an ATPase-dependent mechanism contributes to overall clearance.[5] Serum uric acid concentration within the population has a Gaussian distribution, with a typical reference range (95% CI) of 120–420 μmol/l. For an individual, urate concentration is determined by a combination of the rate of purine metabolism (both endogenous and exogenous) and the efficiency of renal clearance. Purine metabolism is influenced by dietary, as well as genetic factors regulating cell turnover. Uric acid is sparingly soluble in aqueous media, and persistent exposure to high serum levels predisposes to urate crystal deposition within soft tissues.[4] All species apart from man and higher apes express urate oxidase, an enzyme responsible for further metabolism of uric acid to allantoin (a more soluble waste product) prior to excretion.[6] In man, the urate oxidase gene located on chromosome 1 is not expressed due to two non-sense mutations.[7] Loss of uric oxidase activity appears to have developed under evolutionary pressure,[7] suggesting that higher serum uric acid concentrations, or reduced urate oxidase may confer important advantages in man.

Uric acid as a risk factor for cardiovascular disease

An epidemiological link between elevated serum uric acid and an increased cardiovascular risk has

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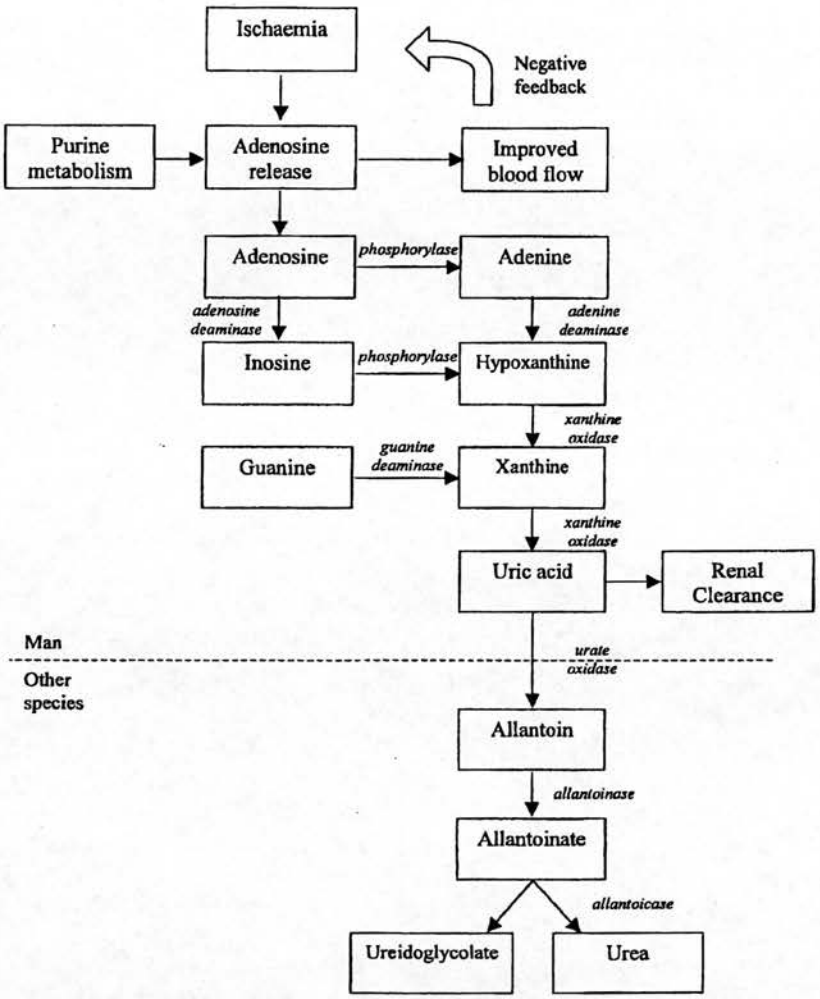

Figure 1. Adenosine release is increased during hypoxia, and its local vasodilatating action may preserve blood flow and prevent tissue ischaemia. Uric acid is the end-product of purine metabolism in man, whereas other species express uric oxidase, responsible for conversion of uric acid to more soluble excretory products.

been recognized for many years.[8,9] Observational studies show that serum uric acid concentrations are higher in patients with established coronary heart disease compared with healthy controls.[10] Elevated serum uric acid concentrations are also found in healthy offspring of parents with coronary artery disease, indicating a possible causal relationship.[2] However, hyperuricaemia is also associated with possible confounding factors including elevated serum triglyceride and cholesterol concentrations, blood glucose, fasting and post-carbohydrate plasma insulin concentrations, waist-hip ratio and body mass index.[3,8,11,12] About one quarter of hypertensive patients have co-existent hyperuricaemia[13] and, interestingly, asymptomatic hyperuricaemia predicts future development of hypertension, irrespective of renal function.[14]

Among patients with established hypertension, elevated serum uric acid concentration has been associated with a significantly increased cardiovascular risk during a mean 6.6-year follow-up period.[15] The proportional hazard ratio for one SD elevation of uric acid (29.2 μmol/l) was 1.22 (95%CI 1.11–1.35), which was higher than for one S.D. elevation of blood glucose (1.10, 95%CI 1.02–1.19), cholesterol (1.18, 95%CI 1.09–1.29) or systolic blood pressure (1.09, 95%CI 1.00–1.19). Thiazide diuretics confer unequivocal benefits in treatment of hypertensive patients, and cause a significant reduction in cardiovascular and all-cause mortality.[16] Persistence of the relationship between elevated serum uric acid concentration and increased cardiovascular risk among thiazide-treated patients has prompted speculation that uric acid elevation may attenuate some of their potential benefits.[17] Indeed, the US National Health and Nutrition Survey (NHANES) III showed that age-adjusted rates of myocardial infarction and stroke

are higher across increasing serum uric acid quartiles among male and female hypertensive patients.[18]

Some studies have suggested that the importance of uric acid may be independent of confounding risk factors. Multivariate analysis of data from the MONICA cohort of 1044 males showed a significant association between raised serum uric acid and cardiovascular mortality, independent of body mass index, serum cholesterol concentration, hypertension, diuretic use, alcohol intake and smoking habits.[19] Comparison of those individuals within the highest serum uric acid quartile ($\geqslant$373 μmol/l) versus those in the lowest quartile ($\leqslant$319 μmol/l) gave an adjusted risk of myocardial infarction of 1.7 (95%CI 0.8–3.3) and cardiovascular death of 2.2 (95%CI 1.0–4.8). The Gothenburg prospective study of 1462 women aged 38 to 60 years also found a significant relationship between serum uric acid concentration and total mortality during 12-year follow-up, which was independent of body mass index, serum lipid concentrations, smoking habit, blood pressure and age.[20] Uric acid also has a predictive role in high-risk patient groups. For instance, diabetes mellitus is a very powerful risk factor for cardiovascular disease, and a prospective study of 1017 non-insulin-dependent patients showed that serum uric acid concentration >295 μmol/l conferred a hazard ratio of 1.91 (95%CI 1.24–2.94) of fatal or non-fatal stroke during 7-year follow-up.[21]

In contrast to these findings, several studies have suggested that the relationship between elevated serum uric acid and cardiovascular risk does not persist after correcting for other risk factors. The British Regional Heart Study of 7688 men aged 40 to 59 years showed a significant association between elevated serum uric acid and fatal and non-fatal coronary disease over a mean 16.8 years.[22] However, this relationship disappeared after correcting for other risk factors, particularly serum cholesterol concentration. The Coronary Drug Project Research Group studied 2789 men, aged 30 to 64 years, and found that the association between increased cardiovascular risk and elevated serum uric acid concentration was not significant after consideration of other risk factors, and when thiazide diuretic use was considered.[23] Similar findings have been reported from the Social Insurance Institution of Finland Study[24] and Framingham Heart Study.[25]

The Atherosclerosis Risk in Communities study of 11 488 healthy men and women showed an apparent association between serum uric acid concentration and early carotid artery atherosclerosis, which was dependent of other coronary risk factors.[26] Similarly, the Honolulu Heart Program found that elevated serum uric acid was not an independent risk factor for the presence at autopsy of aortic or coronary atherosclerosis in Japanese men.[27]

In summary, although there is overwhelming evidence that elevated serum uric acid concentrations are strongly associated with increased cardiovascular risk and poor outcome, prospective population studies are often confounded by co-existent risk factors. It remains unclear whether uric acid is an independent predictor of poor cardiovascular outcome. To unravel this association, it is important to understand the mechanisms by which hyperuricaemia relates to other risk factors, vascular dysfunction and cardiovascular disease. We shall now consider these relationships in more detail.

Uric acid as a marker of subclinical ischaemia

Adenosine is synthesized and released by cardiac and vascular myocytes. Binding to specific adenosine receptors causes relaxation of vascular smooth muscle and arteriolar vasodilatation.[28] Adenosine makes a small contribution to normal resting vascular tone, since competitive antagonism at the adenosine receptor by methylxanthines, such as theophylline, reduce blood flow response to ischaemia in the forearm vascular bed.[29] Under conditions of hypoxia and tissue ischaemia, vascular adenosine synthesis and release are upregulated, causing significantly increased circulating concentrations.[30] Cardiac and visceral ischaemia promote generation of adenosine, which may serve as an important regulatory mechanism for restoring blood flow and limiting the ischaemia[31] (Figure 1). Adenosine synthesized locally by vascular smooth muscle in cardiac tissue is rapidly degraded by the endothelium to uric acid, which undergoes rapid efflux to the vascular lumen due to low intracellular pH and negative membrane potential.[32] Xanthine oxidase activity[33] and uric acid synthesis[34] are increased *in vivo* under ischaemic conditions, and therefore elevated serum uric acid may act as a marker of underlying tissue ischaemia. In the human coronary circulation, hypoxia, caused by transient coronary artery occlusion, leads to an increase in the local circulating concentration of uric acid.[35] Study of tourniquet-induced lower limb exsanguination in patients undergoing surgery shows a five-fold increase in systemic vascular xanthine oxidase activity during reperfusion, and a significant elevation of serum uric acid, which persists for at least 2 h.[36] These findings are also consistent with the inverse relation between baseline serum uric acid concentration and maximal

lower limb blood flow in patients with cardiac failure, where higher concentrations could predict subclinical ischaemia.[37] In conclusion therefore, elevated serum uric acid may be a marker of local or systemic tissue ischaemia and provides one possible explanation for a non-causal associative link between hyperuricaemia and cardiovascular disease.

Uric acid as a marker of insulin resistance

Insulin resistance syndromes result in attenuation of insulin-mediated glucose utilization and confer a substantial increase in cardiovascular risk,[38] through activation of several pathways including the sympathetic nervous system.[39] Elevated serum uric acid is a consistent feature of the insulin resistance syndromes, which are also characterized by elevated plasma insulin level (fasting and post-carbohydrate), blood glucose concentration, and serum triglyceride concentration, and raised body mass index and waist-hip ratio.[2,3] Insulin has a physiological action on renal tubules, causing reduced sodium and uric acid clearance.[40] Despite blunting of the action of insulin on glucose metabolism, sensitivity to the renal effects persists.[40,41] Because plasma insulin concentration is characteristically elevated, hyperuricaemia may arise as a consequence of enhanced renal insulin activity. Elevated serum uric acid concentrations predict subsequent development of diabetes mellitus[42] and hypertension,[14] even in the presence of normal creatinine clearance and plasma glucose concentrations, and therefore may be a subtle, early marker of peripheral insulin resistance syndromes. Thus a link between elevated serum uric acid concentration and cardiovascular disease may arise through its non-causal relationship with insulin resistance syndromes, where cardiovascular risk is mediated by other factors.

Direct impact of uric acid on vascular function

The endothelium plays a central role in maintaining vascular tone through synthesis and release of nitric oxide, a potent vasodilator.[43] Reduction of nitric oxide bioavailability is an important early step in the development of atherosclerosis.[44] So-called endothelial dysfunction, associated with impaired endothelium-dependent vasodilatation may arise from excessive free radical activity, which disrupts synthesis and accelerates degradation of nitric oxide.[45] Thus increased oxidative stress appears to have an important role in development and progression of atherosclerosis and is a characteristic finding associated with its major risk factors, such as diabetes mellitus, hypertension, hypercholesterolaemia and smoking.[44,46] Serum uric acid possesses antioxidant properties, and contributes about 60% of free radical scavenging activity in human serum.[47,48] Uric acid interacts with peroxynitrite to form a stable nitric oxide donor, thus promoting vasodilatation and reducing the potential for peroxynitrite-induced oxidative damage.[49] Thus, uric acid could be expected to protect against oxidative stresses.

However, uric acid has been found to promote low-density lipoprotein (LDL) oxidation *in vitro*, a key step in the progression of atherosclerosis,[50,51] and these effects are inhibited by vitamin C[52] indicating an important interaction between aqueous anti-oxidants. Uric acid can also stimulate granulocyte adherence to the endothelium,[53] and peroxide and superoxide free radical liberation.[53,54] Therefore uric acid may have a deleterious effect on the endothelium through leukocyte activation and, interestingly, a consistent relationship has been noted between elevated serum uric acid concentration and circulating inflammatory markers.[55–57] Uric acid traverses dysfunctional endothelial cells and accumulates as crystal within atherosclerotic plaques.[32,58] These crystals may contribute to local inflammation and plaque progression, and we speculate that crystal accumulation may be greater in patients with elevated serum uric acid concentration.

Thus, while uric acid appears to make a significant contribution to serum anti-oxidant capacity, it could also lead directly or indirectly to vascular injury. It is interesting to note that treatment of chronic cardiac failure patients with allopurinol (a xanthine oxidase inhibitor) restored endothelial function.[59] This effect may have been due to an increase in recorded serum antioxidant capacity,[59] although the effect of uric acid was not considered. Study of the direct effects of uric acid on vascular function has been hampered by its poor solubility, although this has been overcome recently.[60] Direct study of the actions of uric acid on endothelial function, platelet aggregation, vessel wall elasticity and autonomic cardiovascular regulation is required so that its effects on the cardiovascular system and in cardiovascular disease can be determined.

Conclusions

Raised serum uric acid concentrations are a powerful predictor of cardiovascular risk and poor

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